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A Short Course in Practical Virusology
Part I. Methods of Virusological Research
Part II. Brief Data on Specific Virusology.

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Shubladze, S. Ya. Gaydamovich, 1949.

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INDEX OF CONTENTS

Foreword

Introduction

1. General Concept of Viruses and Virus Infections
2. The History of the Discovery of Viruses
3. The Nature and Basic Characteristics of Viruses
4. Antiviral Immunity
5. Epidemiology
6. Prophylaxis and Therapeutics

Part One

GENERAL VIRUSOLOGICAL TECHNIQUE

1. The Isolation of Viruses
2. Purification of the Virus
3. The Study of Carriers
4. The Preservation and Titration of Viruses
5. Filterability
6. The Study of Virus Characteristics
7. Serological Reactions
 - A. The Agglutination Test
 - B. The Complement Fixation Test
 - C. The Neutralization Test
8. The Cultivation of Viruses Outside the Organism of Susceptible Animals
 - A. Virus Cultivation in Tissue Cultures
 - B. Cultivation of Viruses in a Developing Chicken Embryo

RESTRICTED

RESTRICTED

9. Microscopic examinations
10. Differentiation of Viruses
11. Preparation of Vaccines and Sera
12. Laboratory Animals
13. Rules of Procedure for Virusological Laboratories

Part Two

CLASSIFICATION OF SPECIFIC VIRUSES

1. Arthropod-borne Viruses
2. Bacteriophage Viruses
3. Plant-virus Viruses
4. Anthrax Viruses
5. Virus Diseases of Laboratory Animals.

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A SHORT COURSE IN PRACTICAL VIROLOGY

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FOREWORD

The attainments of medical science in its struggle against infectious diseases are growing from year to year. The expansion in the volume and the depth of scientific research brought about the segregation of independent disciplines, which develop the individual aspects of infectious pathology in humans. Virology is one of these independent disciplines, the formation of which is of comparatively recent origin. The reasons for the segregation of virology into a separate branch of medicine are the special features of the research methods it employs. These peculiarities are predicated on the fact that virological diagnostics does not employ the generally accepted in bacteriology methods of breeding in ether cultures in artificial nutritive media and the conventional micro-research.

The segregation of virology into a separate and independent discipline for the first time was accomplished in the USSR and this is, first of all, attested by the creation of the Virological Institute of the Academy of Medical Sciences.

This book attempts a brief summarization of the basic data on research methods employed in virology, with a view of facilitating the study of virus infections for medical personnel, running into the necessity of solving practical problems in their struggle with the infectious diseases of virus origin.

The introductory part and the first part of the book are devoted to general problems and methods in virological research. The second part describes the individual inciters of virus infections

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in humans, grouped by their characteristic of tropicity. The classification of viruses into neurotropic, pneumotropic, dermatropic, and pantropic is rather conditional, and we only follow it for the sake of convenience.

In our labors on this book we were well supported by the staff of the Virusological Institute, Academy of Medical Sciences, USSR, and, particularly, by the following professors: I. A. Zil'ber, V. D. Solovyev, R. M. Shen, M. A. Morozov, V. L. Ryzhkov, A. T. Kravchenko, A. I. Panov, who took upon themselves the labor of checking the manuscript, and were helpful with valuable advice.

The authors.

INTRODUCTORY PART

1. General Conception of Viruses and Virus Infections.

The virus group includes those inciters of infectious diseases, very small in size and located at or below the visibility range of a conventional microscope. Viruses pass through filters, which will entrap bacteria, and cannot be cultivated outside of susceptible living cells. Small size, filterability, and the inability to propagate in artificial nutritive media, are the basic indexes characterizing this group of pathogenic inciters.

Many acute infectious diseases in humans are induced by these peculiar agents: smallpox, measles, mumps, ^{infection}grippe, encephalitis, poliomyelitis, and others. Animals and birds become afflicted with virus diseases as frequently as humans, and some of these diseases assume great economic significance, such as the ^{influenza}grippe in hogs.

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encephalomyelitis in horses, encephalitis in sheep and other animals, hoof and mouth disease, distemper and rabies in dogs, plague, smallpox, infectious laryngotracheitis, fowl sarcoma, and peitacosis in birds.

Virus diseases are widespread not only in humans, animals and birds, but also in plants. Well known examples are the mosaic disease in tobacco, sugar cane, and sugar beet, also the potato disease. Altogether, there are about 200 known varieties of virus infections in plants.

It must be noted that some scientists relegate the bacteriophage to the category of viruses that destroy bacteria.

In their epidemiological significance, the virus infections hardly lag behind the bacterial infections. The epidemic of gripe sometimes involves hundreds of thousands and even millions of people. It is sufficient to recall the pandemic of 1919 - 1920, when more than a quarter of the entire population of the globe were stricken. Even in non-epidemic years, the gripe is the most widespread disease, and is one of the main causes for the temporary work incapacitation of the populace. To this can be added such a frequently occurring infection as measles, and also diseases with a grave course and high mortality, such as poliomyelitis, virus encephalitis, rabies, and a series of other virus diseases.

Virus infections are known for as long a period as bacterial infections. Throughout ancient and medieval history, epidemics of smallpox resulted in tremendous devastations. Traces of smallpox and poliomyelitis were found on mummies, dated to a period three thousand years before our era.

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In Latin the word "virus" stands for venom, and it was originally applied to venoms of animal derivation. Subsequently, it was applied to the designation of any infectious principle. At present, however, the word "virus" is used in a narrow sense only, for the designation of a certain group of infectious disease inciters. Out of various designations suggested, such as ultramicrobes, inframicrobes, ultraviruses, filterable viruses, and viruses, the two last ones attained the widest application.

2. The History of the Discovery of Viruses.

The history of virusology dates back somewhat over half a century, and its beginnings were laid in our country. In 1892, a Russian scientist, the botanist D. I. Ivanovskiy [photo in text], in the course of studying the mosaic disease of tobacco, discovered that the sap of the infected leaves, after passing through a finely porous filter, did not lose its infection-producing properties, and, when carried onto a healthy plant, did not fail to induce infection. Such filtrate did not contain any microscopically visible bacteria and its plantings onto nutritive media remained sterile. The report by Ivanovskiy, on his observation, to a session of the Russian Academy of Sciences, is considered the official discovery date, pertaining to filterable viruses. It should be mentioned, however, that as early as 1886 the oldest fatherland microbiologist, N. F. Gamaleya, took note of the fact that the blood of a cattle-plague infected calf, after having passed through a Chamberland filter, remained contagious to the healthy calves. But, since he had no opportunity for repeating the test, the observation escaped notice.

The Ivanovskiy tests with the virus of tobacco mosaic were again duplicated in 1898 by Beijerinck with the original results fully confirmed. During the same year, Leffler and Fresh discovered that the cattle hoof and mouth disease is caused by a filterable virus. Subsequent years witnessed a series of discoveries in, and the establishment and study of virus inciters. In 1901 the yellow fever virus was isolated; in 1904 Horrel described the elementary corpuscles of fowl smallpox, recognizing these corpuscles as the inciters of the disease. In 1909 Landshtainer and Fopper established that poliomyelitis is induced by a filterable virus and that it can be transmitted to monkeys. Particularly extensive research began after the 1919 - 1920 pandemic of grippe, when the accelerated attention of scientists was drawn to the phenomena of virus infections.

3. The Nature and Various Characteristics of Viruses.

From the time of the first discoveries in microbiology, the days of Pasteur and Koch, the theory of the animate nature of infection agents prevails. As a result, the scientists, coming into contact with a new variety of pathogenic inciters that were filterable, assumed that they were up against animate microbes of an extraordinarily small size. These concepts, however, were shaken after some plant viruses were isolated in their pure form. In 1935, Stanley isolated the tobacco mosaic virus in its pure form by ammonium sulfate precipitation. Ryzhkov used another method for purifying the virus of tobacco mosaic which was based on its adsorption by benzoic acid. The plant viruses, thus purified, turn out to be, by their chemical composition, nucleoproteins of high-

molecular weight, capable of crystallization. Out of 200 known plant viruses about 10 were isolated in crystalline form. However, it is still unknown how these nucleoproteins are propagated in the stricken plants.

[Photo] Academician Emeritus N. F. Gamaleya. Collaborator of Professor I. I. Mechnikov in the organization and guidance of the first Pasteur Experimental Station in Russia, author of a series of treatises on smallpox, rabies, encephalitis, grippa, and antiviral immunity.

Progress in the study of the biochemistry of viruses resulted in the indubitable proof that if the disease inciters in plants are, by their chemical composition, nucleoprotein complexes, the inciters of virus diseases in humans and animals, at any rate, the largest in size, which are known under the name of elementary corpuscles, are very close to bacteria. It is known that the chemical composition of the vaccine virus is almost analogous in composition to a bacterial cell. The chemical analysis of vaccine virus particles showed the presence of nucleoprotein, hydrocarbons, and lipoids. Amongst the lipoids, determinations were made for cholesterol, neutral fat, and chemically combined phosphorus, while the nucleic acid belongs to the desoxyribose type. The discovery, in the vaccine virus, of biotin, flavin-adenine-dinucleotide, and copper, leads to the assumption of the presence in it of enzymes.

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Viruses, like bacteria, carry a negative electric charge, and by the method of cataphoresis can be concentrated in neutral virus suspension on the positive pole.

The problem of the origin of viruses remains unsolved, and existing theories, as yet, lack factual confirmation. Do viruses represent a now existing likeness to the primitive pre-cellular forms of life, being a peculiar and complex variety of albumen, capable of propagation and mutability, or do they stem from microorganisms of the bacterial type? These questions cannot, as yet, be answered. There is no doubt, however, that viruses are the minutest forms of life, which, as a result of continuous evolution, have adapted themselves exclusively to parasitic maintenance within the cells of the susceptible host.

This intracellular parasitism in which the viruses, having lost the capacity for independent metabolism, are accomplishing the latter at the expense of the cells of the macroorganism represents the general nature of the virus disease inciters in animals and plants and is their characteristic biological index.

There is adequate proof that as between the relatively large and the minute members of the virus group there is an essential difference.

For the last 20 years considerable data as to the size of viruses has been accumulated. The largest human and animal viruses visible under a microscope with the aid of special coloring belong to the group of so-called elementary corpuscles. The first one to describe these elementary corpuscles was Borrel' in 1904 while he was studying skin lesions in fowl smallpox. Pashen, after a thorough

study of similar corpuscles in human smallpox (1906), insisted that these corpuscles were the cause of the infection. These formations are now known as the Pashen corpuscles. It was discovered later that these virus elementary corpuscles are also present in other diseases: smallpox of animals, venereal lymphogranuloma, infectious ectromelia in mice, psittacosis.

The sizes of the elementary corpuscles are determined by the Bernar microscope method, the virus being photographed in ultraviolet rays at predetermined magnifications. The dimensions were being compared with the dimensions of the Pashen corpuscles. Ultraviolet photography extends the visibility limits of the conventional microscope more than twice and provides for the obtaining of the images of particles 0.05 - 0.1 μ in size. It is also suggested, for the study of virus particles, to use fluorescent dyes, such as primulin, with subsequent observation in ultraviolet light.

The discovery of the electron microscope provided for the further expansion of the visibility limits. The electron microscope, in the place of beams of light, utilizes electron beams. Electron as well as light beams have undulatory characteristics, it being the case that an electron wave length is about 100,000 times shorter than a blue light wave length. Since the resolving power of a microscope is in an inverse ratio to the wave length of the penetrating rays, the use of electron rays brought into the field of visibility much smaller particles. The electron microscope provides for an image magnified 200,000 times. The virus images practically obtained, however, are magnified 30,000 - 40,000 times.

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In construction the electron microscope is very similar to the optical microscope. As in the case of the optical microscope the magnification is obtained with the aid of lenses which represent magnetic fields. The electron rays, in passing through the magnetic field, are deflected from their original rectilinear direction and the image of a point in the object is located further away from the optical axis than it actually is. Upon refraction through the second magnetic lens, the final image of the object is projected onto a fluorescent screen where it can be observed and also photographed.

Filtration methods, in addition to microscope methods, are widely used for the measuring of virus particles. The diatomaceous earth filters (Berkefeld filters) or filters from non-burnt clay (Chamberland filters) effected an adequately successful separation of viruses from bacteria but could not separate viruses one from the other and determine their sizes.

In addition there were errors due to the adsorption of viruses on the considerable porous areas of the filters. Elford suggested that thin colloidal membranes with uniform pores be used. By filtering through a series of membranes with pores of specific sizes, the dimensions of viruses may be determined with approximate precision.

In addition to the microscope and filtration methods, the centrifuge method is also used to determine the dimensions of viruses. In determining virus dimensions with the aid of velocity centrifuges with a high number of revolutions per minute the

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conformity between the precipitation rate of the particles and the time of the centrifuging operation was taken into account.

Table 1 depicts the comparative dimensions of viruses determined by microscope and photographing in ultraviolet light, by ultrafiltration, and by centrifuging.

Virus	Diameters of Virus Particles in μ		
	Microscopy and photography in ultra-violet light	Ultra-filtration	Centrifuging
	(1)	(2)	(3)
Psittacosis	300	230-330	-
Sheep smallpox	-	170-260	-
Vaccinia	150	125-175	170-180. 210-230
Powl smallpox	160	125-175	120
Veneral lympho granulo-	-	125-175	170-185
fibrous tumor in rabbits	-	125-175	170-180
Herpes	-	100-150	160
Ectromelia	135	100-150	-

10 (a)

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	(1)	(2)	(3)
Rabies (fixed virus)	-	100-150	-
Pseudo-rabies	-	100-150	-
Horn diseases	-	85-120	-
Grippe	-	80-120	-
Newcastle diseases	-	80-120	-
Houss X ? / sarcosis	-	75-100	70
Vesicular stomatitis	90	75-100	-
Fowl Plagues	75	60-90	88
Phage Staphylococcus phage	-	60-90	-
Lymphocytic chorio-meningitis	-	40-60	-
Fevers of the Rift valley	-	25-35	35
Papillomas in rabbits	-	25-35	-
Encephalitis St. Louis	-	22-23	-
Encephalitis (Japanese)	-	21-30	-
Encephalomyelitis in	-	-	-
horses (American)	-	21-30	-
Encephalomyelitis in	-	-	-
birds	-	20-30	-

	(1)	(2)	(3)
Yellow fever	-	18-27	18-30
) Encephalitis in sheep	-		
) (Scottish)	-	15-20	23-27
Tobacco mosaic	-	15	-
Poliomyelitis	-	8-12	-
) Hoof and mouth	-		
) disease.	-	8-12	17-20

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It should be underscored that the greatest precision is obtained by the use of the method of ultrafiltration through graduated membranes.

As to the measuring of virus dimensions with the aid of an electronic microscope, the available data generally confirm the observations made by the method of ultrafiltration.

In considering the basic characteristics of viruses, it is important to note that the elementary corpuscles are not entirely uniform in size or shape. This non-homogeneity is predicated on the characteristics of the virus proper and depends upon conditions of propagation, degree of accumulation and the peculiarities of its existence.

Special coloring methods suggested by Pashen, Morozov, Turvich, and Shen, also electron microscopy, are used in the study of the structure and form of viruses.

Data obtained by the morphological study of viruses confirm the similarity of the elementary corpuscles to bacteria and show that they possess morphological symptoms which are characteristic of live cells (Figures 1 and 2).

Figure 1. Elementary corpuscles of natural smallpox. Contents of skin smallpox pustule. Silvering, as per Morozov. Magnified 1000 times (Morozov preparation).

Figure 2. Elementary corpuscles of the vaccine virus. Electronic shadow photography. Tubercular protrusions

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on the surface are the elementary corpuscles magnified 23,000 times.

Histological methods of examination of virus stricken tissues showed the presence of particular formations which received the name of inclusions. They were first described by Henderson and Peterson in 1941 in the course of study of endothelium cells which were infected by a disease known as Molluscum contagiosum. After that, inclusions were also found in the cases of other virus diseases, such as smallpox, rabies, herpes, windpox. The inclusions vary in size and shape. The experimental formations of inclusions is studied through artificial contamination of animals. For instance, in the case of Herpetic keratitis in rabbits, the inclusions appear within 24 hours after contamination. The inclusions are located inside the nuclei and in the protoplasm of the cells. The origin of the inclusions is, as yet, not sufficiently clear, and seems to be non-homogeneous. Some scientists believe that these inclusions are a particular variety of cell mutations; others consider them to be a stage in the development of the elementary corpuscles. Most of the scientists lean toward the second assumption and today the inclusions are most frequently considered to be accumulations of elementary corpuscles forming, in a manner of speaking, virus colonies.

Regardless of the lack of proof in the homogeneity of origin of these inclusions, their presence in the tissues is considered a symptom of the infection, and, as such, is used for the diagnosis of some virus diseases. It is long since known that in rabies the

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presence in the cerebral tissues of inclusions, known as the Negri corpuscles, is an indubitable symptom of the disease (Figure 3).

Figure 3. [Photo] Intracellular inclusions in the cerebral tissues in the case of rabies -- Negri corpuscles.

The impossibility of cultivation in artificial nutritive media, as was already pointed out above, is a general characteristic of all viruses. Not one virus was ever cultivated in the absence of living tissue. Outside of the living organism, it is possible to cultivate viruses only in various tissue cultures. The use of tissue cultures was first suggested by Karrel in 1913. He obtained growths of tissues, in vitro, from almost all organs of warm-blooded animals: the heart, the kidneys, the liver, the spleen, the thyroid gland, and he developed the technique of the continuous restoration of the nutritive medium during the cultivation. By the regular restoration of the nutritive medium, the destruction of the live tissues in the culture from the accumulation of pernicious exchange products is prevented.

In 1913 the Russian scientist Krontovskiy established that the source of energy in the tissue cultures is furnished by the splitting of ^{carbohydrates} ~~hydrocarbons~~. The first attempts at virus cultivation in tissue cultures produced no results of any practical importance. In 1928 Meyland suggested the ~~method~~ ^{method} of virus cultivation in surviving tissues which obviates the necessity for the presence of multiplying cells.

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The method most widely used at present for the cultivation of viruses is the method proposed by Gudpaschur and Vudruf in 1931, i.e., the cultivation of viruses in a growing chicken embryo. This is the medium in which most of the viruses are now cultivated.

Still other methods for virus cultivation were proposed. Degkviia cultivated the measles virus in association with some bacteria cultures, and Zil'ber discovered the phenomenon of symbiosis between some viruses and yeast fungi.

The study of filterable viruses is largely predicated on the availability of a suitably susceptible animal. A successful selection of an animal most suitably susceptible to a given virus insures the successful study of individual infections. Without going into further detail we refer ourselves to Table 2 where some collated data on animals most susceptible to various viruses is given.

With relation to some viruses, the circle of susceptible animals is wide, as is the case in rabies, human encephalomyelitis, horse encephalitis, smallpox. With relation to some other viruses, the circle of susceptible animals is considerably narrower. Frequently, a given virus will strike only a given species of animals.

Only humans are susceptible to the viruses of "Papstachi" (papstachi fever), Dengue fever, and infectious hepatitis.

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Table 2

Virus	Most Susceptible Animals
Encephalitis St. Louis	Mice, monkeys
Encephalitis Japanese	Mice, monkeys, sheep
Far East spring-summer encephalitis	Mice, monkeys, sheep, field mice
Scottish sheep encephalitis	Mice, monkeys, sheep
Rabies	Mice, monkeys, dogs, cats, rabbits
Poliomyelitis	Monkeys, cotton field mice [rats]
Lymphocytic choriomeningitis	Mice, guinea pigs, monkeys
Herpetic encephalitis	Mice, rabbits, guinea pigs
Acute human encephalomyelitis	Monkeys, rabbits, rats, mice
Psittacosis	Mice, birds (parrots, pigeons, chicks)
Human gripe	Mice, rats, skunks
Gripe in swine	Mice, rats, skunks, pigs
Pneumonia in mice	Mice
Infectious head cold	Chimpanzee monkeys
Smallpox vaccines	Rabbits, guinea pigs, calves sheep, asses
Hoof and mouth disease	Cattle, guinea pigs
Ectromelia in mice	Mice
Measles	Monkeys
Yellow fever	Monkeys, mice
Venereal lymphogranuloma	Mice

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A sharply pronounced characteristic of viruses is their tropism to certain animal tissues and organs. By their tropism, or affinity to individual cellular systems in the organism, viruses are divided into various groups: dermatotropic, which strike mainly at the skin and the mucous membranes (smallpox, infectious ectromelia in mice, hoof and mouth disease, herpes); pneumotropic, which strike at the respiratory organs (human grippe, swine grippe, virus pneumonia in humans, mice, and rats); neurotropic, which cause sickness of the central nervous system in humans and animals (poliomyelitis, rabies, lymphocytic choriomeningitis and a whole series of encephalitis); and pantropic viruses. Into the latter group are conditionally included those inciters of virus infections which do not induce a sharply pronounced lesion of some individual organ, and, with relation to which, there is no proof of predominant propagation in some individual cellular systems (measles, mosquito fever, Dengue fever, yellow fever). To this group we also relegate viruses which are capable of selectively striking the internal organs, as, for example, the infectious hepatitis virus which strikes selectively at the liver, the venereal lymphogranuloma virus, which strikes at the lymphatic glands, and the mumps virus, striking at the salivary glands.

As already mentioned, the classification of viruses by their tropism index is rather conditional, and we follow this principle for the sake of convenience only since it facilitates the systematic presentation of data. It is necessary to emphasize that tropism in viruses is not a permanent and immutable characteristic. If, in the

- 16 -

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group of neurotropic viruses, the initiators have the characteristic of propagating predominantly, or exclusively, in nerve tissue, such selectivity is not necessarily the rule with relation to other viruses. Thus, the herpes virus, belonging in the dermatropic group, when introduced into an animal, selectively strikes at the nervous system and proceeds to propagate in it. The smallpox virus may adapt itself to the brain of rabbits and acquire neurotropic characteristics. The virus of venereal lymphogranuloma, which we included conditionally in the pantropic group, is sustained, under laboratory conditions, in the intracerebral passages of white mice. The virus of yellow fever, in the experimental contamination of white mice, finds its optimum conditions for propagation in the brain, thus acquiring stable neurotropic characteristics.

The course by which any virus reaches a sensory tissue in susceptible animals, propagates selectively in it, and induces pathological changes, has a very essential significance since it determines the pathogenic characteristics of the viruses. The basic course for the dissemination of viruses in the organism is predominantly hematogenic, and the blood, at any time interval, may contain a considerable amount of virus. However, in the case of some neurotropic viruses, there is another course of dissemination along the nerves.

The question on the dissemination of the poliomyelitis virus was under considerable debate. Its dissemination was considered possible ~~along the axis-cylinders of the nerves or along the~~ perineurial lymph. It is difficult to trace the progress of viruses in an organism, yet numerous tests are undertaken in which, with

- 17 -

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- 17 -

RESTRICTED

the aid of virological and pathomorphological methods, it becomes possible to establish the presence of the virus, its progress, or traces of changes in the tissues that were induced by it.

Of no less importance is the establishment of the paths of separation of the virus from the organism and the ascertaining of its stability with relation to external influences. It is a known fact that some viruses become separated through the nasopharynx, or with the urine, or excrement. The saliva of an animal stricken with rabies is very contagious. The grippé virus is easily discovered in the respiratory passages. There is available data to the effect that the virus of poliomyelitis may be found in nasopharynx secretions or, even more frequently, in the stool of stricken humans. Sewage waters in infected localities, in some cases, also contain the virus, it being the case that the virus of poliomyelitis has stability to chlorine. Therefore, the small amounts of chlorine used for decontamination will not affect the virus of poliomyelitis in a $1\frac{1}{2}$ hour chlorination, and only after 4 hours of chlorination does the virus become inactivated. The virus of spring-summer and other transmissible encephalitises is very unstable to the effect of high temperature and to the effect of alkaline solutions. The study of the ability of various viruses to withstand external influences, such as high and low temperatures, radiation energy, various chemical reagents, is instrumental in the establishment of one of the basic characteristics of viruses, their viability in external media. This also creates the possibility of finding ways and means for rendering the viruses harmless.

- 18 -

RESTRICTED

4. Antivirus Immunity.

The practical utilization of immunity, in the case of virus diseases, with the prophylactic end in view, has, by many years, overtaken the theoretical development of our knowledge in this field of the science of infectious diseases. The remarkable discovery by Jenner, who used the virus of smallpox in cows for the prevention of natural smallpox in humans, was made almost a century before the existence of filterable viruses was proved. Vaccination against another virus disease -- measles -- was developed by Pasteur and his disciples, also in the "pre-virus" period.

These discoveries served as the base for the modern structure of immunology which now includes the least known subdivision, the theory of immunity with relation to virus diseases.

Antivirus immunity has its peculiar features which are predicated on the specific characteristics of viruses. In principle, however, its rules differ little from the rules of antibacterial immunity, since, in both these cases, the immunities are merely different manifestations of the same homogeneous biological process -- the defense of the organism against the penetration of an infectious inciter.

In the case of ~~virus~~ diseases, as well as bacterial, the sickness leaves after itself an immunity of various intensities and durations, dependent on the nature of the infection endured. In smallpox, measles, poliomyelitis, yellow fever, virus encephalitis, the immunity after the infection endured is highly effective and may

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be considered lifelong. In other words, secondary attacks, in the case of these diseases, are very rare. In the case of other virus diseases, such as Dengue fever, pappatasii [mosquito] fever, grippa, the post infection immunity is of relatively low duration and intensity, and for herpes and head cold, immunity is practically absent. The antigenic qualities of viruses are analogous to the same in bacteria. Serological reactions, which disclose specific antibodies in vitro, are almost completely coincident in both virus and bacterial diseases. Agglutination, precipitation, complement fixation, and neutralization antibodies, are described in a series of virus diseases. In virus diseases, as well as in bacterial ones, artificial immunity can be induced with the aid of prophylactic vaccinations prepared from live as well as dead virus.

In the nonsusceptibility to virus diseases, whether it be of postinfection or postvaccination origin, the basic manifestation of immunity will be the presence of specific antibodies circulating in the blood. Their significance as a factor upon which antiviral immunity is predicated is not yet adequately clear. There is, however, no doubt that in a series of infections, such as yellow fever, spring-summer encephalitis, these antibodies are a fully reliable index of immunity. Nevertheless, in the case of poliomyelitis, which leaves a postrecovery stable immunity, the antibodies in the blood seemingly do not play any part. There are observations to the effect that, in the case of convalescents, it is not possible to detect any increase in the titration standard of the antibodies in the process of convalescence, and that the blood in patients with an acute phase of poliomyelitis may contain a considerable amount of antibodies which do not impede the

- 2 -
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development of the sickness.

In studying the regularities of immunity in rabies-vaccinated humans, and in the experimental study of rabies in animals, it was discovered that, in rabies, the humoral antibodies are not a determining factor in immunity.

Recent experiments showed that, in addition to the antibodies circulating in the fluids of the organism, there also exist cellular antibodies located directly in the zone of pathogenic action of the inoculum. In the opinion of Mosakovsky, such cellular antibodies may be prime-fixed (sessile antibodies), i.e. antibodies evolving within the cells, or reiterative-fixed cells from the blood or lymph.

Thus, in monkeys recovered from poliomyelitis, the neutralizing antibodies are discovered in the cells of the forward cornucopia of the spinal cord while at the same time they are absent in the cortex of the brain and present, in small numbers only, in the blood.

In rabies-vaccinated mice, a suspension of cerebral tissue possesses a more pronounced neutralizing activity than a serum prepared from the blood of the same animals. It is obvious that, in the above infections, the principal part is played by the sessile antibodies. In the case of encephalomyelitis in horses, it was also established that immunity to secondary contamination is determined by the presence of antibodies in the central nervous system proper, the latter, however, being dependent on the humoral antibodies. The antibodies appear in the central nervous system only then, when the titration standard of the antibodies in the blood is high.

- 21 -

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The particular manners in the manifestation of immunity in individual virus infections are the result of the diversity in pathogenesis. If, as in the case of poliomyelitis, the humoral antibodies in the blood do not play a perceptible part in immunity, this does not constitute proof of their negative significance in other infections, since the virus of poliomyelitis possesses a particularly pronounced tropism to nerve tissue. In the case of spring-summer encephalitis, the inciter of which belongs to the same category but is relatively neurotropic, and, penetrating into the organism hematogenically, it strikes not only at the organs of the central nervous system but also at other tissues, the antibodies in the blood are, as was already pointed out, a reliable index of immunity.

In the case of grippé, the inciter of which enters the organism through the respiratory tract, immunity is, to a considerable extent, conditioned upon the presence of antibodies in the nasal secretions. Francis and Colev'yev established that the antibodies in the nasal secretions are identical with serum antibodies and their numbers are in conformity with the titration standard of the antibodies in the blood.

At this time some scientists (among them Kravchenko) are of the opinion that immunity may exist without antibodies, and, in that case, it would be based on the nonsusceptibility, or inactivity, of the cells. According to this theory, the sensory cells, upon having been penetrated by the virus, alter their reaction to it and the process of infection runs under conditions of changed reactivity.

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In studying antiviral immunity, attention was paid, in addition to the antibodies, to other factors which also play an important part in antibacterial immunity, such as phagocytosis and the reticulo-endothelial system.

Viruses may be subject to fixation by various cells, including leucocytes. Research by Zil'ber, Shetoldayeva, Shubladze established that the virus of herpetic encephalitis and ectromelia in mice, will, in individual cases, be adsorbed by leucocytes with the virus remaining intact.

[Photo] L. A. Zil'ber -- one of the founders of the first, in the USSR, special laboratories for the study of human and animal viruses; the author of many works on the etiology of virus diseases and antiviral immunity.

In observing the phagocytosis of the virus of vaccine in vitro, it was noted that the leucocytes entrap the elementary corpuscles of the vaccine, while the virus, inside the leucocytes, not only does not lose its activity, but is even propagating and forming colonies. On the basis of available research, the authors assume that phagocytosis plays no part in antiviral immunity.

Investigations devoted to the study of the part played by the reticulo-endothelial system are not numerous, but predominantly point to the fact that it is not particularly important insofar as it may be judged on the basis of the disruption of the normal physiological function of the reticulo-endothelial system by blockade

RESTRICTED

and splenectomy. For the formation of antiviral antibodies, the reticulo-endothelial system is as important as it is in the case of bacterial infections, i.e., as generally accepted, the antibodies are formed in the cells of the reticulo-endothelial system.

The denial of the significance of phagocytosis in antiviral immunity led to the question as to how a naturally nonsusceptible organism rids itself of a virus. In bacterial diseases, it is, specifically, phagocytosis and the reticulo-endothelial system that are of decisive importance. To find the answer to this question, it became necessary to study the distribution of the virus in the organism of susceptible and nonsusceptible animals.

It was discovered that in rats, which are nonsusceptible to the virus of herpes, mice ectromelia (Zil'ber, Shubladze), Japanese and spring-summer encephalitis, the virus appears regularly in the urine (Shubladze, Neustroyer). In contaminating frogs with the virus of grippe (Zil'ber, Baydakov), the virus was discovered regularly in the kidneys.

Analogous results were obtained by the study of the fate of the virus in an immunized organism. If these results are brought into conformity with the opinion of the majority of scientists to the effect that the antibodies do not affect the virus directly, but are combined with the cells, it may then be assumed that the susceptible cells, protected by the antibodies, do not effect the fixation of the virus, and the latter is discharged with the urine. Zil'ber believes that the discharge of viruses with the urine from a nonsusceptible organism may be one of the factors of antiviral immunity.

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A basic characteristic of the group of filterable viruses is, as was already mentioned, their intracellular parasitism. In entering the organism, the viruses penetrate the cellular barriers, become embedded in the cells and, by their reproduction, cause the gradual destruction of the cells (necrosis). Specifically this characteristic, i.e. intracellular parasitism, stipulates the peculiarities of antiviral immunity expressed predominantly in the so-called phenomenon of "interference", or the competitive blockade of the cells. In some infections the virus may come in contact with a cell which, instead of being stricken, becomes nonsusceptible to the penetration of a more virulent inoculator. Such a phenomenon was described during an experimental study of a series of virus diseases of animals and plants.

In 1935, Nagasaki showed that the contamination of rabbits through the corneal introduction of the nonencephalitogenic stem of the virus of herpes does not lead to the manifestation of a pathogenic process. However, on the sixth day after the inoculation the virus can be discovered in the brain of the animal. If, four days after the primary inoculation, a virulent virus stem is introduced into the brain of the same animals, the latter will still not be stricken. Such nonsusceptibility of tissue is strong and durable.

The phenomenon of mutual interference was described in the case of yellow fever. Rhesus monkeys, contaminated subcutaneously or intra-abdominally with a mixture of two stems of the virus yellow fever, may be protected by a neurotropic stem from a lethal infection with a pantropic stem. The presence of interference was

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proved as between the various stems of poliomyelitis which were adapted to mice and monkeys.

The phenomenon of mutual interference between viruses does not depend on the formation and effect of antibodies, or on the presence of some other antiviral substance, since it can be observed, not only within an organism, but also in a culture of tissues in vitro, and also in the case where an embryonic membrane of a chicken embryo in the process of development is inoculated with two various stems of the virus grippé.

To date, there is no explanation for this phenomenon which differs considerably from the established phenomena of antibacterial immunity. It is entirely probable, however, that the phenomenon of interference plays a considerable part in the development of nonsusceptibility of the organism to a series of virus infections.

5. Epidemiology.

At the present time, more than 35 infections of humans with an established virus etiology are known. This number is only somewhat lower than the number of infections caused by bacterial agents. The evolution of virus diseases, their propagation and abatement are subject to the same regularities that are known for infections of a bacterial or protozoic nature. A certain peculiar feature in the epidemiology of virus diseases in humans can be seen from the fact that the intestinal tract, as a place for the localization of the infection, is of relatively little importance. Also characteristic, and conditioned on the general

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nature of all viruses, is their intracellular parasitism. The latter determines the greater propagation of the latent forms of infection or the characteristic of virus carrying.

With relation to virus infections in humans, it is only in the case of poliomyelitis that the intestinal tract is the locus, where the virus may be preserved for a long time, and the intestinal tract has a no less important part in the propagation of the disease than the nasopharynx. Recent investigations confirm the opinion that the gateway for the infection is the nasopharynx as well as the gastrointestinal tract. The fact that the virus of poliomyelitis is discovered, during epidemics, in sewage waters brings this disease of the central nervous system, in an epidemiological sense, closely together with intestinal infections of a bacterial nature. It may also be mentioned that the epidemic outbreaks of poliomyelitis are seasonal (summer-autumn).

It is possible that in still another virus disease in humans, namely infectious hepatitis, the above cited peculiarities of poliomyelitis are repeated to some extent. It is possible to experimentally contaminate humans by introducing filtered gargles from the nasopharynx of, as well as filtered feces from the intestines of patients stricken with hepatitis. There are on record outbreaks of hepatitis of water and food origin. However, to date, no virus diseases of the gastrointestinal tract, resulting in pathological changes, are known.

The existence of a protracted virus ~~carrying~~ ^{emerging} period was proved experimentally in the cases of many virus diseases. Such

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a condition, however, cannot always be separated from a chronic infection or an infection which is manifested in atypical and light forms. There is proof to the effect that humans are contaminated with the virus of herpes in early childhood, most frequently from their parents. The virus embedded in the cells of the mucous membranes of the oral chamber is preserved for a long time, possibly throughout the lifetime of the individual. The virus, if propagating, does so only to the extent that is adequate for its survival in the cells. When coming under the influence of various and numerous provocatory herpetogenic factors, it becomes pathogenic, and, with rapid propagation, induces the formation of herpetic vesicles. In epidemics of poliomyelitis not more than a fifth of all cases are being diagnosed as typical with a pronounced infection of the central nervous system. The remaining cases are atypical light forms or nonparalytic poliomyelitis. There are on record experimentally proven cases of virus carrying on the part of outwardly healthy people who were in close contact with a stricken patient.

A classical example of an infection, which is durably surviving in an organism, is the infectious anemia in horses. A horse may be contaminated with the virus of infectious anemia, and without showing any symptoms carry the virus in its blood for a long time. The blood of such a horse, over a period of years, is capable of inducing a lethal sickness in healthy horses. In some cases, the contact of humans with such horses resulted in contamination with the symptoms expressing themselves in heavy enteritis, general asthenia, and emaciation.

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From the blood of one such patient, over a three-year period, after the clinical symptoms had disappeared, the virus was segregated twice and its potency proved by contaminating horses with it. In psittacosis (virus disease of parrots), the infection may be transferred from the stricken bird to humans, resulting in a case of heavy, frequently fatal, pneumonia. At times the infection is communicated directly from a human stricken with psittacotic pneumonia. Forty such cases are on record, most of which resulted in death. However, such a transfer of psittacosis from human to human is rare, and only birds may be considered the true carriers and propagators of this virus.

Most frequently the young fledglings in the nest become contaminated with many of them remaining virus carriers for a long time. Thus, out of the number of 200 wild parrots captured in various regions of Australia all were outwardly in good health. However, laboratory tests established the fact that 50 percent of them were carriers of the psittacosis virus. It was also established that it is a widely prevalent disease not only among wild parrots but among other birds as well, particularly pigeons.

Out of all virus diseases, there can be segregated a group of transmissive infections with blood sucking carriers. In the case of other groups, where nonvertebrates do not appear to be the transmitters of the virus, contamination occurs by direct or indirect contact.

A large group of viruses, to which humans are susceptible, were incorporated by Pavlovskiy under the general name of transmissive viruses. They are transmitted to humans when the latter

RESTRICTED

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are bitten by various transmitters. These viruses and their transmitting agents are enumerated in Table 3 below.

Table 3

Virus	Transmitting Agent
Spring-summer encephalitis	Tick Ixodes persulcatus
Scottish encephalitis	Tick Ixodes ricinus
Japanese encephalitis	Mosquitoes Culex tritaeniorhynchus, Culex bitaenioides, Aedes albopictus, Aedes togoi.
Horse encephalomyelitis	Mosquito Culex tarsalis
Encephalitis St. Louis	Mosquito Culex tarsalis
Encephalitis (Western Nile)	Mosquito Aedes albopictus
Mosquito fever	Mosquito Phlebotomus papatasi
Dengue fever	Mosquito Aedes aegypti
Yellow fever	Mosquito Aedes aegypti

Transmissible virus infections are characterized not only by the presence of a blood sucking transmitting agent but also by the particular conditions in nature upon which the survival of the transmitting agent is predicated. For instance, the ~~propagation~~ boundaries of transmissible infections coincide precisely with the ^{of occurrence of} propagation area for the transmitting agent, ^{thus explaining a seasonal character of the infection} by the virtue of which the endemic character of the infection is ~~constituted~~. The period of maximum reproduction of the blood sucking transmitters of the virus in their ^{the infection local} breeding places coincides in time with the period of maximum rise in morbidity. This explains the rigidly pronounced seasonal character

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Seasonability of transmissible infections. The end of spring and the beginning of summer is the maximum morbidity season for tick-induced spring-summer encephalitis which is preceded by the maximum reproduction period in the life of the transmitting agent for this virus, the tick *Ixodes persulcatus*. In the case of Japanese encephalitis, the maximum morbidity season occurs with the arrival of autumn, i.e. with the season abounding in mosquitoes.

In the epidemiology of the transmissible virus infections, in addition to the blood sucking transmitters, of considerable importance is the part played by the wild animals inhabiting the breeding places.

It was established that the virus of spring-summer encephalitis survives in the ticks throughout the winter and can be transmitted transovarially to the new progeny, which makes the ticks not only the transmitting agents for the virus but also its natural reservoir. However, the reproduction and metamorphosis of the ticks are closely tied in with their parasitism on animals. The female tick, having attained puberty, lays her eggs only after satiation with the blood of mammals, and the larva, hatched from the egg, is transformed into a nymph after feeding on small rodents or birds. The nymphs, too, feed on rodents, but may also attack larger animals.

The virus of spring-summer encephalitis was found in the organism of some wild animals captured in the endemic breeding places. The animals included field mice, chipmunks, moles, and hedgehogs. The virus is not transmitted directly from the animals

- 31 -

RESTRICTED

RESTRICTED

to humans, but the part played by the animals in the dissemination of the virus is indubitable. The virus is transmitted from the contaminated tick to a mammal, and from the latter, in its turn, new contingents of ticks are contaminated.

Birds probably serve as the reservoir for the virus of American horse encephalomyelitis. The separation of the virus from chicks, pheasants, and the discovery of antibodies in the serum of a great many animals corroborate such a possibility. The probability of the existence of a virus reservoir in birds has also been noted with relation to Japanese encephalitis.

In a large group of sicknesses the transmission of the infection occurs by direct contact, which is the case in venereal lymphogranuloma, with contamination through conventional warts. The virus of rabies is transmitted by contact with the saliva of a stricken animal. The dripping discharge route for the transmission of infection is observed in the spreading of such diseases as measles, epidemic grippa, and infectious head cold. The usual route of smallpox contamination is through the nasopharynx. Until now it was thought that poliomyelitis was transmitted by the dripping discharge route. However, the discovery of the virus in the stool of stricken patients, the successful contamination of monkeys through the feces from patients, and other observations, shook the concept on the routes of infection for poliomyelitis. It is now considered that the gastrointestinal tract, too, is the source of the spread of the infection and possibly its entering gateway.

- 22 -

RESTRICTED

RESTRICTED

Humans are sometimes contaminated through contact with stricken animals or with animals who are virus carriers. Such contaminations are known in the cases of lymphocytic choriomeningitis and psittacosis.

In studying the epidemiology of virus infections, the methods applicable to bacterial and protozoic diseases are used. They are accumulated by establishing: the nature of the epidemic; the geographical dissemination of the infection; the widespread or limited dissemination by zones, regions, cities, villages; the effect of population density; the predominance of morbidity by months and years; the seasonability, as to spring-summer, autumn, winter; the effect of climatic conditions; the tie in with other infections; the count of secondary infections and the immunity-possessing segment of the population; the determination of the incubation period; the count of overall morbidity, as well as of the age and sex of the stricken individuals; the presence of worn-out and abortive forms of the infection; the extent of virus carrying by the sick and the healthy; and, finally, the ascertaining of the method of propagation and the source of the infection.

6. Prophylactics and Therapy.

Prophylactics and therapy in virus diseases is the most difficult and least developed area in virusology. As of today, the following prophylactic measures are known:

- (1) General sanitation and hygiene.
- (2) Various methods for combating the carriers of virus

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infections. In this category belong measures undertaken against the preparation of mosquitoes, such as the drying of swamps and the destruction of mosquito breeding spots with the aid of various insecticides, the destruction and the repelling of winged mosquitoes by the use of strongly odoriferous compounds, screening of doors and windows, and individual means of protection (special clothing, strongly odoriferous salves and liquids). In combating ticks the following measures are used: the clearing of undergrowth, spraying of individual areas with insecticides, the use of specially designed clothing, regular tick removal inspection of people at work.

(3) Specific measures designed to combat a particular infection. In this category belong vaccination and seroprophylactics. Vaccination is used for the purpose of preventing diseases that are capable of epidemic dissemination. Antivirus vaccines are mostly a suspension of tissues or organs from an infected animal, or a suspension of tissues of a virus-contaminated chicken embryo, which were subjected to inactivation by phenol or formalin. There are also vaccines in which the virus was inactivated in a different manner, such as ultraviolet vaccines, photodynamic vaccines, heated vaccines, etc. Formalin vaccines found practical use in the prevention of spring-summer, Japanese, horse encephalitides, and grippa.

The difficulty of obtaining effective vaccines for the prevention of virus infections is due to the fact that the inactivated virus frequently loses its immunogenic characteristics. Experience points to the fact that the best vaccines are live vaccines in which the virus has not been inactivated but has so changed in its

RESTRICTED

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initial properties that it has lost its infectivity, retaining, however, its immunogenic properties. There are three such vaccines: antismallpox, antiyellow fever, and antirabies, the latter being rather a therapeutic preparation.

Live vaccines against smallpox and yellow fever give durable and strong immunity. However, the attempts at the modification of the viruses, for the purpose of obtaining such vaccines, were rarely successful. This is pointed up by the fact that from the days of Jenner and Pasteur only one more live vaccine (anti-yellow fever) was developed successfully. The viscerotropic virus of yellow fever, which is lethal to monkeys, after numerous passages through mice by intracerebral contamination, has acquired neurotropic characteristics and lost its pathogenic qualities with relation to humans and monkeys.

Such a modified virus, in the form of a culture on a chicken embryo, is used for immunization against yellow fever in the epidemic areas of Africa and America. Research in the field of obtaining active compounds is being continued in the direction of modification of the viruses, with the view of obtaining live vaccines and raising the effectiveness of inactivated vaccines by the methods of their purification from inert substances (tissue albumens, fats) and virus concentration. In this respect the investigations, directed toward the obtaining of new varieties of live antiviral vaccines, hold out the best prospects.

This field holds adequately wide possibilities for the practical realization of the theory of heredity by Michurin-Lysenko.

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Two important, matter of principle, facts, which are known from experience with viruses, must be taken into consideration:

(1) the relatively simple, as compared with other more complex organisms, ability of viruses to change their initial characteristics and acquire new ones as a result of changes in the conditions of their external cultivation medium; (2) the ability of vaccinal virus stems to retain with stability their acquired characteristics and pass them on in a hereditary manner. Observations over a period of years, with relation to the vaccinal stems of the viruses of smallpox and rabies, fully confirm this postulate.

For prophylactic purposes a specific serum is sometimes used. In accordance with the existing concept, the antibodies in the serum from reconvalescent or immune animals dispose themselves on the surface of the susceptible cells, hindering thereby their penetration by the virus. A preliminary introduction of the serum, as shown by experiments with various virus infections, forestalls the sickness, yet its prophylactic effect is not too durable. The introduction of the serum a long time after the contact with the inciter, when the virus has already penetrated into the cell, may produce no results since the virus, already embedded in the cell, is inaccessible to the antibodies [of the serum]. This is why, in the case of measles, the seroprophylactic treatment is effected before the fourth or fifth day of incubation. The use of the serum after this time limit has expired may only soften the severity of the sickness. The use of seroprophylactics in the case of neuroinfections is even less successful, since the serum encounters an impedance in the hematoencephalic barrier which

- 36 -

RESTRICTED

RESTRICTED

makes the access to the central nervous system difficult. However, in the case of neuroinfections, where the virus is disseminated in the body not only by way of the nervous system, but also by the blood current (where the virus becomes accessible to the effect of the antibodies), the use of seroprophylactics is warranted (as in the case of spring-summer and Japanese encephalitis).

Immunity serums are obtained from humans and animals who recovered from the disease, or from healthy animals (horses, asses, dogs) which were especially immunized with respective antigens. For example, in measles the serum of adult humans who recovered from measles is used. To obtain antiencephalitis serums, horses are immunized with a vaccine and, additionally, with the live virus until the titration standard for the antibodies in the blood of the horse attains a high level. All the above serums are defective since, in addition to the antibodies, they contain a lot of inert albumens which makes it necessary to use large quantities.

The production of purified and concentrated serums will overcome the above shortcomings and increase the effectiveness of the serums. At the present time the gammaglobulin fraction of the serum is being successfully segregated, raising thereby the concentration of the antibodies 20 - 25 times.

Therapeutic treatments, as applied to virus infections, add up to serotherapy, vaccinotherapy and chemical therapy. Serotherapy is conducted by taking into consideration the same conditions that

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were cited in the description of seroprophylactics. Consequently, the basic therapeutic condition is the earliest possible introduction of serum in its most purified and concentrated form. However, many experiments in the therapeutic use of immunity serums have failed to prove their effectiveness.

Therapeutic vaccines received recognition in a series of bacterial infections: tularemia, brucellosis, various autovaccines (with pyrogenic manifestations). In virus diseases, this therapeutic method is, as yet, little developed. The only positive results known were obtained in the treatment of patients stricken with acute encephalomyelitis and multiple sclerosis with a formalin-containing vaccine prepared from the virus of acute human encephalomyelitis.

The progress of modern chemical therapy in the field of bacterial infections turned out to be of little use in virology. Numerous attempts at the utilization of various chemico-therapeutical compounds in the treatment of virus infections were not successful. Individual positive results were obtained with large viruses only. The retarding action of sulfidine upon the propagation of the virus of psittacosis in a chicken embryo, which expresses itself in retarding or forestalling the destruction of the embryo, is known. Sulfamide compounds are used successfully for the treatment of venereal lymphogranuloma.

The effect of para-amidobenzoic acid upon the virus of acute human encephalomyelitis, in vitro and in vivo, was tested. Although a one percent compound, after 15 minutes, inactivated the virus in

- 3 -

RESTRICTED

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vitro, the attempt to treat animals, infected with even small doses of the virus, did not produce any effect.

The question as to the therapeutic action of known antibiotics is generally answered in the negative. Penicillin and streptomycin were tested for effect upon the viruses of vaccine, gripe, encephalitides, horse encephalitis, and psittacosis. A weakly positive result was noticed for the viruses of the lympho-granuloma group only, and for psittacosis, when used in very large doses.

Negative results in the treatment of virus diseases are due to the intracellular disposition of the viruses, which condition restricts the effect of even such compounds as will inactivate in a flask virus particles in vitro.

PART 03

METHODS OF VIRUSOLOGICAL RESEARCH

The inciters of virus infections are most frequently discovered in a stricken organism during the first days of the sickness, in the period of the most acutely pronounced symptoms, when the infection is at its climax.

The virus segregating substances are the fluids and tissues of the organism in which the virus is disposed in maximum quantities. In neurotropic infections, such substance is the cerebrospinal fluid and the blood of the patient, and, in sectional cases, pieces from the brain, spinal cord, and peripheral nerves. For the

RESTRICTED

segregation of pneumotropic viruses, the initial substance is the secretion from the upper respiratory passages, separated by gargling the nasopharynx with a physiological solution or with a conventional bouillon. In sectional cases, pieces of the lungs, bronchial tubes, and a mucous pharynx scraping are examined. In dermatropic infections, the segregating substances are the stricken sections of the skin or mucous membranes, the contents of pustules and vesicles, and, in some cases, also the blood. In pantropic infections, the segregating substance is, first of all, the blood of the patient, with examinations also made of the pharynx cavity, cerebrospinal fluid, the urine, and the stool. In infections with doubtful etiology, suspected to be of virus origin, the following substances must be examined (when there are no indications of selectivity in the lesions): the blood, the cerebrospinal fluid, the pharynx cavity, the urine, the feces, and, in sectional cases, pieces of organs and tissues.

1. The Taking of Specimens.

The taking of specimens from patients must be done under rigidly aseptic conditions. To take blood from a vein, the conventional needle injector is to be kept in boiling water for 20 minutes and then cooled in a closed sterilizer. The blood, taken in the amount of 2 - 10 cubic centimeters, is poured off into a sterilized vial containing beads, and, by shaking for 15 minutes, is defibrinized. It is necessary to avoid such defibrinizing solutions as sodium citrate which is not well endured by animals intracerebrally. It is also possible, for the separation of the virus, to use coagulated blood, which, prior to being administered,

RESTRICTED

is triturated in a beater, with the addition of a small amount of physiological solution (1:1, or 1:2). The sterilized containers are to be opened or closed only through the flame of a gas or alcohol burner. When [absorbent] cotton plugs are used, their charring is to be avoided.

A specimen of the cerebrospinal fluid is taken with the aid of a lumbar puncture made by a needle injector, or collected, drop by drop, from a touch needle into a sterilized test tube, with the observance of the same rules indicated for the taking of blood specimens.

The pharynx gargle is prepared as follows: vials containing a sterile physiological solution, or the conventional meat-peptonic bouillon, in the amount of 10 - 20 cubic centimeters, are prepared in advance. It is recommended that 10 percent of normal serum (horse, male goat, rabbit) is added to the physiological solution. The patient is to gargle the mouth and throat thoroughly for not less than one minute with this amount of liquid, and expectorate into the same container. In the case of patients who, for various reasons, are unable to go through with this procedure, their pharynxes are to be swabbed with sterile cotton tampons similar to those used for taking smears from the throat of diphtheria patients. The tampon is then swilled in one of the above indicated solutions, which are distributed, in 2 - 3 cubic centimeter amounts, into sterile test tubes.

The urine is to be taken with the aid of a catheter into a sterilized closed container. The feces is also collected into a

- 41 -

RESTRICTED

RESTRICTED

sterile container, and before using it is subject to a special treatment: a 10 percent suspension of it in a physiological solution is prepared, then allowed to settle for one hour at a temperature not to exceed 5 degrees Centigrade, or centrifuged for 10 minutes at 3000 revolutions per minute, to eliminate the large particles, and, finally, passed through a bacteriological filter. In still other methods, filtering is substituted by high speed centrifuging, or by the elimination of the bacterial flora from the feces with the aid of disinfectants which have no harmful effect upon the viruses. Such are, for example, a 0.5 percent phenol solution, highly diluted mercurio-phenolic compounds, Mercuraphen⁷, etc.

In virological examinations of the central nervous system, the dissection of the encephalon is conducted in a conventional manner, with the avoidance of damaging the hard cerebral membrane. The cerebral membrane, at the section to be examined, is dissected with the aid of sterilized instruments, then, with the aid of a scalpel and pincers, 2 - 3 cubic centimeters of brain are removed. The spinal cord is dissected as follows: with the aid of a sterile surgical saw, individual sections of the vertebral column are dissected, and then, with the aid of a sterile metallic rod having an absorbent cotton tampon on its end, fragments of the spinal cord are pushed out into a sterile container. In order to preserve the membranes, the dissection is done with a cambered double saw. The spinal cord is exposed throughout its length, then specimens are taken from its jugular, pectoral, and lumbar sections. Sections of peripheral nerves are also removed

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with the aid of sterile instruments.

The taking of specimens from the internal organs (the heart, the lungs, the liver, the spleen, the kidneys, the glands) must be done immediately upon the dissection of the respective chambers, ahead of the pathologic-anatomical examination. The area of the exposed organ is cauterized with a red-hot metal spatula, a small piece of the cauterized layer is cut off with a sterile scalpel and discarded, and, from the depth of this section, a 2 - 3 cubic centimeter specimen of the tissue is taken. Glands are peeled out from their capsules. Specimens of the intestines, stomach, or bladder are cut out with the aid of surgical scissors and placed into sterilized containers. It is recommended that the specimens thus obtained be used immediately for the contamination of susceptible animals. If this cannot be accomplished immediately, the specimens are to be placed in a refrigerator for a period not to exceed 24 hours, the temperature not to exceed 5 degrees Centigrade. When solid carbon dioxide, which provides a temperature down to minus 70 degrees Centigrade, is available, the above substances may be preserved for a long time.

For shipping to the laboratory, fragments of organs are placed in a 50 percent glycerin solution in a thermos with ice. To prepare the glycerin solution, chemically pure glycerin is mixed in equal quantity with a physiological solution and sterilized in an autoclave at 120 degrees Centigrade for 30 minutes (the pH value of the solution is brought to 7.2 - 7.6). In placing the fragments of organs into glycerin, it is imperative to make sure that they are completely immersed in it. It must be remembered that in blood,

RESTRICTED

cerebro-spinal fluid, urine, pharynx gangles, and the contents of vesicles and pustules, the virus is subject to rapid deterioration, and the successful segregation of the virus is often predicated upon the rapidity of the examination.

2. The Precipitation of the Virus.

The precipitation of viruses is most frequently accomplished by introducing the substances being tested into laboratory animals or into chicken embryos. For most of the known viruses, there is a selected series of susceptible animals and an established method for their most effective contamination. In the case of neurotropic viruses, the most effective contamination is by way of intra-cerebral administration.

Fragments of the brain or internal organs are preliminarily pulverized in special homogenizers, in jars containing beads, or in beaters (porcelain or glass). In case the tissues in the beater are excessively resistant to grinding, sterilized quartz or river sand may be added. From the ground up organs, a 10 percent suspension in a physiological solution, bouillon, or buffer solution is prepared. Most frequently, a phosphate buffer, consisting of 25 grams of Na_2HPO_4 and one gram of K_2HPO_4 , is dissolved in one liter of distilled water (sterilized in an autoclave). The suspension is centrifuged for the precipitation of the large tissue and sand particles for 10 - 15 minutes at 1500 revolutions per minute, then administered to susceptible animals by one of the above described methods. For intravenous administration, the initial 10 percent suspension is usually diluted 10 more times over.

When there is no assurance that the virus containing substance does not also contain an admixture of bacterial flora, it is administered to the animals only after filtering, or after being treated with a solution of sulfadiazine or penicillin.

For the precipitation of dermatropic viruses with the aid of specimens obtained from patients, animals are contaminated by direct application to damaged skin or mucous membranes. For example, the virus of smallpox is carried onto the scarified skin, and the keratogenic virus of herpes -- onto the scarified cornea of the eye of a rabbit.

The results of virus precipitation are considered positive only in those cases when the animals, after the respective incubation period, develop recognizable symptoms of the infection under study, and when, with the aid of specimens taken from the animals thus stricken, a group of healthy animals is successfully contaminated. These ensuing contaminations are called passages, and by such passages the viruses precipitated from the stricken animals are cultivated and maintained. In the process of these passages it is recommended that the specimens be subject to repeated filtration.

In conclusion it must be pointed out that, in precipitating the viruses, all the available data on the pathogenesis of the infection are to be taken into account, and, in some known virus diseases, only those substances are to be investigated which may unconditionally contain the given virus. The successful precipitation of the virus comes with more ease in the first days of an acute disease and during epidemics, while in individual sporadic cases particularly, not too clear-cut diagnostically, the virus-

ologist is compelled to investigate a variety of substances taken from the stricken individuals, frequently without result. Therefore, the knowledge of the facts established by experience with the precipitation of viruses already known is absolutely imperative. Each virus infection is distinct in its peculiar characteristics, in view of which circumstance practical directions will be given under the respective subdivisions devoted to individual infections.

At present the most extensively applied method for virus precipitation is the introduction of the substance under test into a developing chicken embryo. The procedure for this operation will be described below, in a special chapter.

The periodical occurrence of individual sporadic cases of a disease in the same location over a number of years, or the simultaneous occurrence of many cases of the disease in one or several locations, in the form of an epidemic outburst, with the subsequent occurrence in the same locations of individual cases, or mass outbursts, indicate the presence of an endemic seat of infection. Under such circumstances it is necessary to search for the inciter of the disease not only on the body of the stricken individuals, but also in their environment.

With relation to the discovery of the virus in the environment of the patient, outside the organism of humans and animals, an example of positive discoveries is furnished by investigations made in poliomyelitis when this virus was discovered in the sewage waters in the neighborhood of a hospital in which poliomyelitis patients were treated. During the 1940 epidemic of poliomyelitis

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In the United States, the presence of the virus was discovered in the sewage waters of a series of American cities. The testing procedure was as follows: 4 liters of sewage water collected from the sewer system was allowed to settle for 3 - 4 hours at a temperature of 4 degrees Centigrade. Then 700 cubic centimeters of the liquid from the middle layer was extracted, and 105 cubic centimeters of ester added to it. The mixture was kept overnight at room temperature. In the morning 20 cubic centimeters of this mixture was administered to monkeys intra-abdominally and 200 - 400 cubic centimeters subcutaneously. In the same cities, in a series of tests conducted over a period of 15 months free from epidemic outbreak, the sewage water showed no trace of the virus. During the epidemic period the virus was precipitated from sewage water not only in the cities of the United States but also in Stockholm (Sweden). In most cases the virus was present in sections of sewer systems close to hospitals.

Mention must be made of tests for the precipitation of the virus poliomyelitis from flies, with positive results obtained twice, during an epidemic in two American cities. In both cases the flies, obviously, were in contact with the feces from patients.

Another example of positive results in searching for the virus in the environment of the patient is the precipitation of the virus of lymphocytic choriomeningitis. In the home of one stricken individual two gray domestic mice were captured and the virus precipitated from their organism; in the home of another one, 3 mice were captured and the virus precipitated from the organisms of 2 of them. On the other hand, 21 mice captured in houses that

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were free of the sickness showed no presence of the virus. The researchers who conducted these tests (Armstrong, Smith, and others) maintain that the gray mice are the seat of this virus. Subsequently the data on gray domestic mice as the seat of the virus of lymphocytic choriomeningitis received confirmation; particularly when a batch of 128 mice, captured in dwellings, were tested with 52 percent of this number revealing the presence of the above virus.

In the case of infections, relative to the propagation of which there is available data on the part played by blood sucking carriers, the search of the environment for the factor adds up to investigations of the state of spontaneous contamination with the virus on the part of insects and other arthropoda assembled at the endemic source, and to the precipitation of the virus from wild animals and birds. In such a manner the spontaneous (natural) state of contamination with the virus of spring-summer encephalitis on the part of ticks *Ixodes persulcatus* was established. The spontaneous state of contamination with the viruses of horse encephalomyelitis and encephalitis St. Louis on the part of mosquitoes was established. In the study of the carriers of the above two diseases, over 15,000 arthropoda were collected in the Joachim Valley (United States) and they were tested for the presence of viruses. Three stems of encephalitis St. Louis virus and five stems of western horse encephalomyelitis virus were precipitated from the organisms of the *Culex tarsalis* coquillett mosquitoes. Thus, proof was obtained that the above variety of mosquitoes are the carriers of encephalitis St. Louis and horse encephalomyelitis viruses in America. In the case of all the other varieties of blood sucking arthropoda [In the above collection] the results

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of the tests were negative.

In a series of tests on mosquitoes collected in areas of the Far East, where cases of Japanese encephalitis were observed in 1931, four strains of the Japanese encephalitis virus were precipitated by Sergeev, Shul'zke, and Giazin. The next year, Petrishcheva and Shul'zke, by extensive observations, ascertained the species composition of the mosquitoes in the endemic seats of encephalitis, their state of spontaneous contamination, and their ability to retain and to transmit the virus to animals under experimental conditions. They established the presence in the endemic seat of encephalitis of 26 species of mosquitoes capable of attacking humans. For a state of spontaneous contamination, 51,000 mosquitoes belonging to 11 different species were tested: *Anopheles hyrcanus*, *Aedes vexans*, *Aedes caspius caspius*, *Aedes caspius corsalis*, *Aedes esoenalis*, *Aedes riparius*, *Culex modestus*, *Culex varans*, *Culex tritaeniorhynchus*, *Culex bitaeniorhynchus*, and *Culex pipiens*. The virus was precipitated from the organisms of only two species: *Culex tritaeniorhynchus* and *Culex pipiens*. In 1940, Petrishcheva and Smorodintsev precipitated the virus from mosquitoes *Aedes esoenalis* and *Aedes togoi*. In Japan the virus was precipitated from mosquitoes *Culex tritaeniorhynchus* collected in their natural habitat.

The state of spontaneous contamination with the viruses of yellow fever and Dengue fever on the part of mosquitoes *Aedes aegypti* was established. In Pappatasii fever the virus is carried by the mosquito *Phlebotomus pappatasi*.

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A thorough analysis of the regularities in morbidity occurring in the endemic seat and, principally, of the endemicity and seasonal cycles entailed points to the necessity of investigating the blood suckers predominant in the stricken areas. Such an analysis during an epidemiological study of spring-summer encephalitis made it possible for Zil'ber to advance and prove the theory of the transmission of the above sickness by ticks and to assume that the tick *Ixodes persulcatus*, predominating in the above area, is the carrier of the virus. As is known, this theory was confirmed by all subsequent investigations and, at the present time, is generally accepted. Thus, it can be seen that the necessity of studying the blood sucking carriers of the virus must be based on preliminary epidemiological observations.

3. The Study of Carriers.

In the study of ticks, mosquitoes, and other blood sucking carriers, suspected of being transmitters of the infection, immediate shipping of the collected creatures to the laboratory, or the organization of field laboratories, becomes necessary.

The collection of ticks in their natural habitats calls for specially designed clothing -- overalls, hermetically sealing off the neck, wrists, and ankles. The head must be protected by a white bandage with a loosely descending netting over the neck and shoulders, impregnated with repellent mixtures. It is recommended that no less than two people venture out on such an expedition. Every 30 minutes they are to stop, thoroughly examine each other, and remove the ticks which found their way into the outer clothing and are crawling toward the exposed parts of their bodies. All

- 56 -
RESTRICTED

RESTRICTED

these precautions are particularly important in encephalitis unsafe areas where the possibility of contamination through ticks is very great.

The ticks are picked off the grass and undergrowth by dragging a piece of white material or gauze through the latter. The ticks detained by the material are clearly visible against the white background. They are carefully removed with the aid of pliers, or by rubber gloved hand, and placed in a thick glass tube which is plugged by a tight-fitting cotton tampon covered with thin white material or gauze. Ticks are also picked from the bodies of grazing livestock or captured wild animals. In these cases special care is to be taken in order not to crush or damage the ticks.

All subsequent manipulations with the ticks are done in boxes or in a specially segregated room. In all the work connected with transferring the ticks, particularly the small and squirming larvae, portable boxes are to be used. The test tubes containing the material are put into a test tube rack on a tin tray, or into a white enameled vessel, the edges of which are covered with a sticky paste to detain the ticks about to crawl out. The material is taken apart over trays, the edges of which are covered with a sticky paste, or in jars which are placed in a large vessel filled with a lysol solution. A count of all the captured ticks is taken, and a distribution by species is made. Adult ticks imago, females and males, nymphs, and larvae are segregated. The empty ticks are maintained separately from the saturated ones. The saturated females, collected from animals, are placed separately into special test tubes

- 51 -

RESTRICTED

RESTRICTED

adapted for egg laying.

The ticks are maintained in small Erlenmeyer flasks, or test tubes with a sterile sawdust or sand litter at the bottom, and a piece of accordion-like folded filtering paper which is moistened daily with 5 - 7 drops of sterilized water (Figure 4). The flasks and test tubes are plugged up with a tight-fitting cotton tampon which, in turn, is covered tightly with white gauze. Until the time of the virological examination the tick material is maintained at room temperature. If long period storage is called for, the material is placed in a refrigerator at 5 degrees Centigrade. The ticks can be safely maintained over rather long periods at low temperatures. When transferred into an enclosure, where higher temperatures are maintained, they rapidly come out from the state of anabiosis, with an insignificant number of them perishing as a result of the temperature change.

Virological examinations are conducted by the use of two methods: the method of preparing suspensions and the method of stinging. In the first case the empty adult ticks are distributed into several groups with not more than 30 in each group. The saturated females are examined individually or in groups of 5. The number of empty nymphs and larvae may reach 500 - 1000, the number of eggs (an entire laying) -- several thousand. The saturated larvae and nymphs are distributed into groups of 30 - 50 each. Each group is placed into a sterile test tube which is flooded with 2 - 3 cubic centimeters of ester for a 5-minute period. After several shakings, the ticks perish rapidly under the effect of the ester vapors, with their body surfaces scoured.

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RESULTS

Upon removing the ester with the aid of a Pasteur pipette, the group of ticks is washed 5 times in 10 cubic centimeters of a sterile physiological solution. Then, with the aid of a flat scalpel or a cambered spatula, the clean ticks are collected into a sterile beater covered with a gauze jacket. In the beater, the ticks are thoroughly triturated for 15 minutes, with the addition, by drops, of a sterile bouillon or a physiological solution. For each sample group of 30 empty ticks, one cubic centimeter of solution is added. If the sample group consists of 15 ticks, one half of a cubic centimeter of the solution is used, and so on. Saturated ticks are diluted in a 10 percent solution by weight.

The tick suspension is then drawn off from the beater into centrifuge test tubes and centrifuged for 15 - 30 minutes at 3000 revolutions per minute. The clear liquid over the sediment is administered to [Susceptible] animals to test the presence in it of the pathogenic virus. For instance, the suspension of the ticks *Ixodes persulcatus*, collected from the forests of the Far East, were introduced into the brain of healthy mice, resulting frequently in encephalitis. In other cases, the exposure of minimum amounts of the virus called for additional passages of the brain tissue to fresh animals.

The usual procedure is three consecutive passages of the brain tissue from primarily contaminated mice. If 1000 empty adult ticks are collected, they should be distributed into 33 groups, 30 ticks in each group, and from each group no less than 3 mice (more is still better), or altogether at least 99 mice are to be contaminated. Another group of 99 mice is required for the

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second passage, and still another group of 99 mice for the third passage. Altogether 297 mice must be available for this experiment. If all the mice, during all the three passages, remain healthy, with the exception of those that perished due to non-specific causes, the results are recorded as negative. In those cases, when during the first passage all mice are stricken with the appearance of identical clinical symptoms (for instance, paralysis of the posterior extremities) and, during the second passage from these mice, three fresh mice are stricken with the manifestation of identical symptoms, the result of the test is recorded as positive, the precipitated virus is sustained by further passages to fresh mice, is preserved in glycerin, and is subject to an all around virological study.

In most cases, during the first passage only 1 - 2 mice are stricken, without the manifestation of clear clinical symptoms. From such stricken mice a separate series of passages is undertaken and a positive result is registered later on when the typical regularity of clinical manifestation of the disease in the contaminated animals asserts itself.

The state of contamination with the virus on the part of the ticks is also determined by the sting method. Let us cite the experiment from the operating procedure in the study of spring-summer tick encephalitis. The mouse is affixed onto a table with its back upward (Figure 5). The test tube containing the ticks is applied with its open end to a spot on the skin of the back, which is cleared of hair, for a half hour, or hour, after which the mouse, with the adhering ticks, is removed from the stand. A collar made

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of celluloid or tin is affixed to the mouse, after which she is placed in a wide-necked jar covered at the edges with a sticky paste. The jar is covered with a wire netting and put into a water-filled basin. The animal and the litter are inspected daily and the saturated ticks, which have fallen off, are removed. The attachment of more than five adult ticks is to be avoided, since the stings of a greater number of ticks become toxic to the mice and may result in the death of the animals. When experimenting with ticks in the nymph or larva stage, the mouse is placed in a glass jar covered with a sticky paste at the edges. The nymphs and the larvae are thrown into the jar. After 24 hours, the mouse with the attached larvae or nymphs is transferred to a clean jar, while the parasite material remaining in the first jar is flooded with lycol. When nymphs are fed on the mouse, a ring collar is affixed on the mouse to prevent the throw-off of the ticks and their tails devoured by the mouse. Saturated larvae and nymphs, and sometimes ticks, fall away; they are picked out from the litter and cotton at the bottom of the jar. The mouse, on which the ticks fed, whether still healthy or stricken after 8 - 9 days, is put to death, and its cerebral tissue passed into fresh mice.

Ticks are investigated by the stinging method not only on mice, but also on rabbits. The procedure is as follows. An accurately counted number of ticks, contained in a bag of thin white material, is placed on a shaved area of the rabbit's back. A thin tin disc is placed around the neck of the animal to prevent the possible scratching off of the ticks (Figures 6 - 7). The cage containing

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the mice or the rabbit is put into a container with water. The saturated ticks, which have fallen off, are picked out and counted. If the count does not tally, the cage, the litter at the bottom, the surface of the water in which the cage stood, are thoroughly searched. The litter of the cage and the cage are decontaminated. The animals are further observed and examined (by passages or blood tests for the content of antibodies, etc.).

The collection of mosquitoes is conducted by drawing gauze sacks over the surface of the vegetation cover, usually in daylight. The same method is used during summer evenings. During the day, the mosquitoes are collected into special mosquito-catching tubes, with one end apex-concaved inward. The opposite end of the tube is tightly plugged up with a cotton tampon. When the collecting is done, the catch end of the tube is also plugged up with a cotton tampon and the tube taken to the laboratory. During the excursions, the mosquito catching tubes that are already filled are put into a bag protected from the sun.

From the bodies of animals, the mosquitoes are collected with the aid of wide-necked entomological tubes or apex-like end tubes. When necessary to preserve the mosquitoes alive, they are let out into a small netting-enclosed area where they are sorted by species. From these sorting areas they are again captured with the aid of entomological tubes and placed, by species, into individual (species) net enclosures. The enclosures are installed on tin trays.

The working space at the table, on which the collected

- 56 -

RESTRICTED

material is disassembled, is to be covered with a triple layer of gauze saturated with lysol and slightly wrung. From the air sack, the mosquitoes are also picked out carefully with the aid of wide entomological tubes. Special care must be taken to avoid the crushing of some mosquitoes. When a crushing occurs, all the objects that were in contact with the crushed mosquito are to be flooded with a 5 percent lysol solution.

The mosquito larvae are collected in their breeding places (swamps, artificial water reservoirs, etc.), put into water filled glass containers with silt and foliage at the bottom of the container.

As in the case of ticks, the virusological examination of mosquitoes is conducted by two methods: the preparation of suspensions, and the direct sting test.

When the immediate utilization of the collected material for the preparation of suspensions is called for, the collected mosquitoes are put to death with ether, distributed by species, grouped (30 each) into sterile tubes, and subjected to the same treatment as the ticks.

In testing by the sting method, the species assorted mosquitoes are maintained in little net enclosures and fed -- until the beginning of the test -- on sugar-sweetened water (Figure 8). This is done once a day by moistening a piece of sterilized cotton in sugared water, putting it on top of the enclosure, and covering it with a Petri glass. In the same manner, the mosquitoes may be contaminated with a virus, by adding the virus to the sugared water.

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The virus suspension is made by adding fresh defibrinated blood with the sugar.

Experimentation with infected mosquitoes is difficult and dangerous on account of their great mobility and the chance of a mosquito gaining freedom during a careless moment. A method of working with artificially de-winged mosquitoes was successfully tested out by Shlenova.

The state of spontaneous contamination on the part of mosquitoes is tested by the sting method as follows. The wool of the test animal is shorn off on the upper part of the body. The animal's (mouse, rat, guinea pig) body, stretched out and affixed onto a small board, is carried into the net enclosure containing the mosquitoes under conditions conducive to blood sucking (preferably in the evening, a darkened enclosure, and proper temperature and humidity). It is recommended that the mosquitoes be kept hungry prior to the test (sugar syrup to be withheld).

For feeding upon larger animals, several mosquitoes are placed in a jar, the openings of which are tightly covered with fine netting and tightened with a bandage. The jar is applied to the exposed area of the animal's body and the mosquitoes drink the animal's blood through the netting. The mosquitoes can also be fed by pressing the net enclosure containing them directly against the exposed area of the animal's body. The net enclosures and the glassware used are decontaminated with lysol or in an autoclave.

... The animals, which were bitten by the mosquitoes, are observed, and if, on the passing of the proper incubation period,

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there are no signs of morbidity, a series of subsequent passages is conducted.

In order to establish the state of spontaneous contamination on the part of wild animals, they are captured with the aid of special traps or by various other means. The animals killed during the hunt are examined on the spot; the captured ones are shipped to the laboratory. The examination is usually made on the blood and the internal organs of the animal.

The blood is collected from the animal's heart, in a sterile manner, with the aid of a needle syringe. Nice and other small rodents are completely drained of their blood. The test animal, under ether narcosis, is affixed to a small cork table, abdomen upward, the area of the subclavicular trough covered with iodine, the skin coverings are cleared so as to form a sort of pocket. With the aid of sterile scissors, the axillary cluster of blood vessels is dissected. The blood runs down into the pocket formed by the dissected skin, from where it is drawn off with sterile pipettes. Internal organs are extracted under aseptic conditions and suspensions are prepared from them for the purpose of contaminating susceptible animals. When impossible to examine the material on the spot, also in long distance transportation to the laboratory, the material is to be kept at a temperature not to exceed 5 degrees Centigrade, with the test organs immersed in a 50 percent glycerin solution. Material from several animals of the same species may be combined. The species of the animals and places of entrapment are to be carefully recorded.

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In studying the state of spontaneous contamination on the part of animals in their endemic breeding places, not only the direct method of testing for virus carrying, but also blood tests for the presence of specific antibodies are conducted. Thus, in studying the endemic centers of spring-summer encephalitis, not only was virus carrying on the part of wild animals established, but also the wide propagation of virus-neutralizing antibodies in the blood of wild and domestic animals of the area came into view. In this case, the antibodies in the blood of the animals are an index of the virus antigen entering the body of the animals through bites by virus-carrying ticks.

4. The Preservation and the Titration of Viruses.

Upon precipitation, the virus must be stored, preserved, and maintained in the laboratory. Passages (consecutive contaminations of susceptible animals) promote the propagation of the virus and the intensification of its pathogenic characteristics, which calls for the most conducive method of contamination, bearing in mind the type of tropism inherent in the particular virus. The passages of neurotropic viruses are conducted in the following manner: contaminated animals, stricken, as per schedule conformant with the incubation period, and manifesting clear and typical clinical symptoms, are esterified or chloroformed to death. Their carcasses are then drenched in a 5 percent lysol or phenol solution and placed in a pan or upon a cork table for dissection. The dissection instruments are sterilized in boiling water and, after each manipulation, they are burned through in an alcohol burner flame. The skin is cut open in the occipital area and separated away from the surface of the head

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with the aid of small scissors (Figure 9). With a surgical scissors held in the left hand, the head is gripped through the eye socket while, with the aid of the above scissors held in the right hand, the head is severed from the body and passed through the flame of the burner. With another pair of sterile scissors the cranium top plate is dissected, the brain is extracted and put into a sterilized Petri cup. From the brain of each animal, with the aid of sterilized scissors, a small piece is cut away and placed into a test tube containing a sugar bouillon for the control of bacterial contamination. The culture is placed in a thermostat for a day, and the cup containing the brain is placed in a refrigerator. The next day the cultures are recorded, and the brain, the number of which corresponds to the tube with the blotted bouillon, is centrifuged, and the sterile material (brain) is collected into a sterile jacket covered beaker, or into a jar with beads, where it is pulverized into a 10 percent suspension in a physiological solution or bouillon. The suspension is centrifuged, and 3 - 6 fresh animals (first passage) are contaminated with it. A small amount of the suspension (0.01 - 0.05 cubic centimeters) is used for a bouillon culture for the repeated control of bacteriological sterility.

The brain of the animals stricken through the first passage becomes the material for the second passage, etc. The brain of animals, which died in the experiments, is not to be used since there is a rapid disintegration of tissues right after death.

For conventional passages, mice are used, since they are susceptible to most of the neurotropic viruses. If the virus comes

- 61 -

RESTRICTED

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through several (5 - 6) passages, infecting animals, which have the same incubation period and homogeneous clinical symptoms, it is then recorded as a definite virus stem, is sustained by further passages, and its ability to survive, and other characteristics, are studied.

During the continuous passages of individual stems, the pathogenic characteristics of the inoculum and its numbers in the brain are increased, but only up to a certain limit. The number of viruses is determined by titration. The minimum virus dosage, which is still capable of causing the death of an animal, is called the titration standard of the virus.

Titration is effected in the following manner. Tenfold accelerated dilutions of the brain suspension of stricken animals, from 10^{-1} to 10^{-6} (the titration standard of the virus is rarely higher), are used. From each dilution a group (not less than four) of mice is contaminated. The dilution, which causes the death of 50 percent of the test animals, is considered to be the titration standard. This dosage became known as the "end point" of 50 percent lethality, or LD₅₀. It is difficult, however, to produce dilutions, one of which will coincide exactly with the titration standard of the virus.

Several methods for the computation of LD₅₀ were developed.

At the present time the most widely used are the method of statistical count and the method produced by Reed and Metch. These authors recommend incorporation into the test of a large number of groups of animals, each group containing 5 - 6 animals. The

RESTRICTED

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deviation from the true value of LD₅₀, which is predicated on the number of test animals being small, will be reduced with the greater number of test animals, since, in computing the percentage of lethality, the data on cumulative lethality is taken into account. Let us analyze the example as given in Table 4.

Table 4

Virus dosage (in dilution)	Number of Animals in each solution		Cumulative data		Percentage of Lethality
	survived	perished	survived	perished	
	A	B	C	D	
10 ⁻¹	0	6	0	25	100
10 ⁻²	1	5	1	10	95
10 ⁻³	2	4	3	10	82
10 ⁻⁴	2	4	5	10	66
10 ⁻⁵	3	3	8	6	42
10 ⁻⁶	4	2	12	3	20
10 ⁻⁷	6	0	18	1	5
10 ⁻⁸	5	1	23	1	4

In column C, against the figure showing the lowest degree of dilution (10⁻¹), the number of mice surviving the test, as per column A, is cited; against the figure 10⁻², the number of mice surviving the given and preceding dilutions, and so on, taking into account that each mouse that survived the lower degree of virus dilution would in all probability survive a higher degree of dilution. In column D, against the figure showing the highest degree of dilution (10⁻⁸), is cited the number of mice perished, as per

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column B; against the dilution 10^{-7} is the number of mice equal to the sum of the ones perished under the given (10^{-7}) dilution and the preceding (10^{-6}) dilution of the virus, and against each subsequent dilution is the number of mice equal to the sum of the ones perished under the given dilution and all dilutions which are higher than the given one. This, then, is what we call cumulative lethality since it can reasonably be expected that if a mouse perishes under a 10^{-8} dilution, it will certainly perish under a higher virus concentration. In column L, the percentage of lethality for each degree of dilution is computed as per the following equations:

$$\text{Percent of lethality} = \frac{100 \times \text{number of perished (as per Column D)}}{\text{sum of perished and survived (as per C and D)}}$$

$$\text{Percent of lethality at } 10^{-1} = \frac{100 \times 25}{25} = 100;$$

$$\text{Percent of lethality at } 10^{-2} = \frac{100 \times 19}{20} = 95;$$

and so on.

As can be seen from Table h, there is no dilution under which the lethality is exactly 50 percent. Consequently, the titration standard of the virus is somewhere between 10^{-4} and 10^{-5} . The dilutions, under which the percentage of lethality approximates 50 percent [from both directions], are called the highest and the lowest critical doses. For the final computation of LD₅₀, two formulas are proposed: the lethality value in our example varies in an inverse ratio to the logarithm of the dilution.

- 64 -

RESTRICTED

This distance ratio (X) can be computed from the following equations:

$$X = \frac{\% \text{ of lethality under highest critical dose} - 50\%}{\% \text{ of lethality under highest} - \% \text{ of lethality under lowest critical dose}}$$

$$\text{or } X = \frac{66-50}{80-42} = \frac{16}{38} = 0.56$$

On the basis of a series of experimental observations it became known that, in the titration of any one preparation for use on animals, the percentage of lethality increases in direct ratio not to the absolute values of the test doses, but to their logarithms. Therefore, the value of the dose LD₅₀ can be computed as follows:

$$\lg LD_{50} = \lg 10^{-4} + 0.56 \times \lg 10 (\text{multiplicity of dilution})$$

$$\lg LD_{50} = \dots 4 - 0.56 \times 1$$

$$\lg LD_{50} = \dots 4.56$$

The antilogarithm of this value = 1:45700, i.e. the requisite dilution of the virus, making LD₅₀ = 1:45700.

The passage of pneumotropic viruses is effected as follows. If the initial virus containing material, introduced intranasally, infects the animals (usually mice or skunks), the material for the next passage will be the lungs of these animals. The lungs are extracted under thorough aseptic conditions (Figure 10). The animal is put to death by esterification and stretched out, abdomen upward, paws fastened to a cork or wooden board. The paws of small animals (mice, rats, guinea pigs) are fastened with pins; the paws

RESTRICTED

of larger animals (rabbits, skunks, etc.) are tied to the operating table. The pectoral and abdominal areas are wetted with a 5 percent lysol or phenol solution. With a pair of scissors that were boiled in water, a longitudinal cut is made through the skin from the center of the abdomen to the neck of the animal, after which the skin is separated to the left and to the right. The area exposed is gently rubbed through with alcohol and passed through a flame. Then, two longitudinal cuts are made in the ribs from the diaphragm upward while the thorax is gripped by the breastplate, with pincers held in the left hand. Folding the separated part of the thorax away to the right, the lungs are gripped with sterile pincers at the base of the bronchial tubes, undercut with small scissors, extracted, and placed into a sterile Petri cup. In the case of mice, the heart frequently comes out with the lungs. Immediately upon being placed in the Petri cup, the heart is cut away and discarded, the lungs are rinsed in 5 cubic centimeters of physiological solution and carried with the aid of sterile pincers into a porcelain beater covered with a 3 - 4 layer gauze jacket. In the beater, the lungs are triturated for 10 minutes, with the addition of physiological solution or bouillon, until a 10 percent suspension, corresponding to the weight of the lungs, is obtained. In order to arrive at a fine suspension, it is necessary to add a small amount of quartz sand.

The average weight of the lungs of a mouse is 0.25 - 0.3 grams; therefore, 2.5 - 3 cubic centimeters of physiological solution or bouillon are added per single lung. It is recommended that the Petri cup be weighed prior to the placement of the lungs,

RESTRICTED

and then with the lungs in it. The difference is the weight of the lungs. The lung suspension obtained is centrifuged for 5 minutes at 1500 revolutions per minute. Such a suspension serves for the intranasal contamination of fresh animals (first passage), after daily control cultures in bouillon and agar-agar are taken.

In pantropic infections, the virus containing material suitable for passages is frequently the blood, sometimes the spleen, liver, or glands of a stricken animal. In all cases, the internal organs are extracted in a sterile manner as described above. A 10 percent suspension is prepared in physiologic solution or bouillon with relation to the weight of the test organ. Several drops of the centrifuged suspension are made into a sugar bouillon or agar-agar culture in petri cups, in order to determine the absence of bacterial contamination on the part of the virus stem designated for passage. In case the growth of bacteria in the bouillon or agar-agar is discovered, the virus suspension is to be filtered, and the contamination effected with the aid of the suspension filtrate.

In the case of dermatropic viruses, the initial materials for passage are usually the transmuted areas of the skin, the fluid from vesicles and pustoles, or the affected internal organs of a stricken animal. For instance, in hoof and mouth disease, the fluid from the vesicles on the mucous membrane of the mouth, pharynx, or on the paws of the stricken animal, is rubbed into a scarified area of the skin of a guinea pig. The latter can also be infected by bringing the virus into contact with a scarified area of the

- 67 -
RESTRICTED

RESTRICTED

mucous membrane on the inner surface of the lips. The first vesicles appear on the test guinea pigs after 24 - 48 hours in the areas of the skin inoculation; then, after 1 - 2 days, the secondary vesicles appear in the areas where no direct inoculation took place, such as the mucous membranes of the mouth, tongue, and on the bottoms of the feet. The fluid from the vesicles of the stricken guinea pigs is reinoculated onto scarified skin areas of fresh guinea pigs, effecting thereby a passage of the virus. Although rabbits are susceptible to the hoof and mouth virus, they are not suitable for the passage of the virus on account of secondary symptoms appearing irregularly; the same is true of white rats. White mice are naturally not susceptible, but field mice take to the contamination with great regularity, and can, therefore, be used for the passage of the virus.

The virus of smallpox is also passed on by rubbing the smallpox material onto the scarified cornea or skin of rabbits. The virus of infectious ectromelia in mice can be passaged by two methods. In one method, the liver of the stricken mouse, usually containing great numbers of the virus, is used. A 10 percent liver suspension is introduced into fresh mice intra-abdominally, in the same manner as was done with other pneumotropic viruses. In the second method the infection material is introduced subcutaneously on the inner surface of the posterior paw of the mouse with the aid of a fine needle in the amount of 0.03 cubic centimeter. After 3 - 4 days, the contaminated paw of the mouse becomes edematous, assumes the shape of a pear, the skin in the area of the edema is thinned out and becomes almost transparent. A small

- 68 -

RESTRICTED

RESTRICTED

prickings or incision reveals an accumulation in the edematous tissue of a clear yellowish fluid. In smears from this fluid, colored by the Vorozov method, a multitude of elementary corpuscles can be discovered. To effect a passage, the mouse is put to death, the edematous paw is cut off and placed for 2 - 3 minutes into ester or a Lysozol solution, and then thoroughly washed in a sterile physiological solution, weighed, and triturated in a beater. Adding physiological solution, a 10 percent suspension is made and centrifuged for 10 - 15 minutes. The suspension is inoculated into the skin of the paws of fresh mice. Paws with damaged, suppurated, or necrotized skin are not used for passages.

We will limit ourselves to the already cited examples, and will merely point out that, in all cases, it is necessary to titrate every virus stem in order to establish its pathogenic characteristics, determine the frequency of virus passages so that its virulence is not diminished, or the virus stem altogether lost.

The titration of various virus stems is conducted as per the diagram cited above with relation to neurotropic viruses, it being the case that the dilutions of the virus containing material are prepared in volumes and quantities to conform with the initially established titration standard of the given virus stem.

In the case of some viruses, frequent passages are required; other viruses can survive the intervals between passages, at low temperatures, in the form of ready to use virus suspensions, or in the form of fragments from organs and tissues immersed in a 50 percent glycerin solution. The preservation of various virus stems

- 69 -

RESTRICTED

RESTRICTED

in glycerin is widely used, and can be successfully applied under any operating conditions (in scientific expeditions and field campaigns). A well passed virus stem in the form of fragments from the brain of a stricken animal (neurotropic viruses), fragments from the liver or the edematous paw of a mouse (virus of eczematella in mice), fragments from the lung of a stricken animal (virus of erippe), etc., is placed into a tube containing 5 - 6 cubic centimeters of a 50 percent glycerin solution (Figure 11).

Figure 11. The preservation of virus-infected organs of a mouse in glycerin. 1 - lungs of mice stricken with erippe; 2 - liver of mice stricken with eczematella; 3 - brain of mice stricken with encephalitis.

A tag is pasted on the tube with the name and number of the virus stem, the time recording, and the sequence number of the passage. A duplicate of the tag is posted in the daily ledger, and the tubes are placed in a refrigerator, the temperature not to exceed 5 degrees Centigrade. Most of the well passed virus stems (5 - 10 passages) survive under such conditions up to several months. The encephalitis viruses, granted a continuous suitable temperature in the refrigerator, can survive in glycerin up to one year or even more, with, however, somewhat lowered pathogenic characteristics.

The initial ultimate titration standard of a virus may be restored in a short time by subsequent passages.

Prior to the test, the virus stems (fragments from organs) that were preserved in glycerin are thoroughly washed in a sterile physiological solution, since contamination with material containing

RESTRICTED

glycerin will sometimes cause subsidiary phenomena, particularly when the contamination is intracerebral.

For the protracted survival of most of the known viruses, the most reliable method is preservation in a dry state. A rapid desiccation at low temperature in vacuum is called for. Apparata of the Flossart-Made type and other laboratory installations are used for this purpose. In a simple laboratory desiccation apparatus a low temperature of minus 70 degrees Centigrade is attained by the use of dry carbon dioxide mixed with alcohol. A cylinder filled with calcium chloride is used for dehydrating, and electrically driven oil pump -- for the creating of a vacuum. A diagram of such a simplified laboratory installation is presented in Figure 12.

Figure 12. Diagram of apparatus for the desiccation of serum and viruses (the Bekkedov diagram).

1 - electric motor; 2 - vacuum pump; 3 - tank with chemical moisture absorber; 4 - manometer; 5 - ampule attachment rack; 6 - refrigerant mixture bath; 7 - vacuum rubber hose connections. Top detail -- connection of the ampule to the rack; 8 - cambered tube; 9 - ampule placed into refrigerant mixture.

The virus that has been fixed by various passages is studied for its pathogenic characteristics with relation to various laboratory animals under various methods of inoculation. The initial virus suspension is used for the contamination of rabbits,

RESTRICTED

RESTRICTED

guinea pigs, rats, mice, and other animals, intracerebrally, intravenously, intra-abdominally, intranasally, subcutaneously, and intracutaneously. Sometimes other methods of contamination are used, such as onto the scarified cornea of the eye, into the anterior eye chamber, into the spino-cerebral canal. In addition to establishing the pathogenic characteristics of the virus and selecting the most susceptible test animals, the adaptability of the virus to new species of animals is studied. In doing so, considerable transmutations in the initial characteristics of the virus are frequently discovered. Thus, the inoculation of a calf with the virus of smallpox, with the subsequent passages of the virus, modifies the virus in such a manner that it becomes harmless and does not induce the disease of smallpox when inoculated into a human being. The passages of the street virus of rabies through the brain of rabbits cause the evolution of a fixed virus, which, even though it kills rabbits more rapidly when introduced intracerebrally than the street virus, is not pathogenic through subcutaneous inoculation.

RESTRICTED

5. Filterability.

In the further study of precipitated viruses, their filterability is established. At the present time, solid ceramic filters, originally introduced into bacteriological procedure by Pasteur and Chamberland, and also colloidal membrane ultrafilters, are used. The colloidal membrane ultrafilters, in particular, came into wide use, beginning with 1931, when a number of scientists developed the method of constructing colloidal membranes to a specified diameter of perforations, i.e. membranes of easily reproducible and precision graduated porosity.

The Chamberland filters are elongated cylinders closed at one end and hollow inside. They are made of Kaolin (hydrous aluminosilicate), with an admixture of quartz sand, and carefully calcined at a low temperature so as not to allow the formation of a compact mass, and preserve the requisite porosity. The Chamberland filters (candles) are made to various degrees of porosity, and are designed as per factory established scale: L₁ - is a filter with the largest pores, and allows the passing of all microbes; L₂ and L₃ detain the large microbes and allow the smallest ones to pass; L₅, L₇, L₉, and L₁₃ are more compact, in the sense of having the tiniest pores, and they detain all bacteria.

The Berkfeld filters, similar in principle to the Chamberland candles, were designed in 1922. They are made from diatomaceous earth, the basic component of which is silicic acid, contained in the armor of diatoms. The several degrees of porosity are designated by the letters V, N, W, in the order of diminution of the density of the filter walls.

RESTRICTED

RESTRICTED

In addition to the above cited filters, there are Mandler filters, containing from 60 to 80 percent of diatomaceous earth, from 10 to 30 percent of asbestos, and from 10 to 15 percent of gypsum, depending on the porosity required. The Kramer filters are prepared from gypsum with an admixture of calcium carbonate and magnesium oxide, - also in the form of candles. In wide use are the Zeyts filters, which consist of laminated asbestos disks of various sizes inserted into special metal holders. Large area plates make it possible to filter a considerable volume of liquid at one time.

Each filter must be specially mounted - adjusted to the receiver, into which the filtrate is collected. The receivers may be simple devices in the form of glass cylinders with side tubules ending in narrow funnel-like bottoms. The drawing off of the air is effected through the tubule by a water jet pump (or any other pump). A rubber tube is attached to the funnel in the bottom. The rubber tube is inserted into a glass tube ending in a sealed up capillary.

Upon filtration, the liquid is distributed from this tube into individual tubes or ampules. Flat bottom conical vessels, with side tubules in their upper part, through which the air is pumped off, are used as receivers. The filter-candle is inserted into a rubber plug, which seals tightly the opening of the receiver. A second opening in the plug is adjusted for the sterile pumping of the filtrate. Metal adapters are used for the mounting of the Zeyts filters.

RESTRICTED

Figure 13. A Zeyts filter mounted and connected to a hand pump.

They are made from nickel plated metal and consist of two parts - the upper cylindrical bottomless part, and the lower part, which is the bottom of the apparatus, and is held in place by special screws. The bottom narrows down at the center into a narrow tubule through which the filtrate is discharged. Between the two parts of the adapters, an asbestos plate, laid upon a metal netting for support, is tightly clamped. The liquid to be filtered is poured into the cylindrical part, from where, as it is filtering through the asbestos plate, it enters the receiver of the mounted filter.

The virus containing suspensions to be filtered are preliminarily freed from coarse particles by centrifuging or filtering through a paper filter. The filtration is most frequently done under negative pressure, which is created with the aid of any pump (water jet, electric, or hand pump). The pressure must not be high (not to exceed 40 centimeters of the mercury column), since a higher pressure may force small bacteria through the pores of the filter. Prior to filtration, the serviceability of the filter is checked by immersing it in water and forcing air through it under accelerated pressure. Under a pressure of one atmosphere, the filter must not pass any air bubbles. After the filter is properly mounted, the plug contacts with the receiver and the filter are to be paraphrased.

RESTRICTED

The serviceability of the filter is also controlled by filtering some smallest visible microbes. The non-passing of the latter through the filter insures the suitability of the filter for virus filtration. The most frequently used bacteria for this test are *Bact. prodigiosus*. After this test filtration, the filtrate is planted onto nutritive media for a sterility check. These plantings are observed over a period of 5 days. Asbestos plates used once are discarded.

Ceramic filters (candles) may serve for secondary filtrations. They are specially treated for the removal of pore clogging particles. At first, they are sterilized for 10 hours with a 3 percent phenol solution, then washed in water and in 2 percent Javel water for two hours, and, finally, scrubbed with a brush in 10 percent hydrochloric acid for two hours. The filter is then washed in running water for 24 hours, to drive off the hydrochloric acid, until the chlorine reaction disappears completely, and left for the night in distilled water. The candles that were washed, are dried in a thermostat. Sterilization is effected in an autoclave. A more radical method of cleansing the candles, mainly, of outside organic particles, is calcination in a muffle furnace.

In recent years, there was introduced the method of ultrafiltration through filters-diaphragms made from materials of organic origin, which allow the separation of colloidal phases in solutions with a high degree of dispersion. Such filters are, therefore, called ultrafilters. Their pores are of pre-determined diameters, and they are also called graded-perforated filters. (See Figure 17)

RESTRICTED

Figure 14. Apparatus for the making of diaphragm ultrafilters (installation of the bio-chemical laboratory at the Institute of Virusology) 1 - regulating table; 2- glass chamber for collodion pouring; 3- level; 4 - protection cover (celluloid); 5 - jars with ready-to-use diaphragms; 6 - flask with collodion solution; 7 - measuring cylinder.

Figure 15. Installation for ultrafiltration - 3 units (as per the Tolarnitskiy diagram). 1- for positive pressure filtration; 2 - complete counting for sterile ultrafiltration; 3- for filtering into a Bunzen flask.

Figure 16. Apparatus for high pressure ultrafiltration.

Figure 17. The pores of colloidal diaphragm filters (as per Graber').

a - magnified 400 times (colored slide);
b- magnified 400 times (non-colored slide);
c - magnified 600 times;
d - pore diameter of 930 millimicrons;
e - pore diameter of 700 millimicrons;
f - pore diameter of 430 millimicrons;
g - pore diameter of 250 millimicrons.
Magnification = 600 times.

RESTRICTED

In the making of grade-perforated diaphragms by the Elford method, the initial material is nitrocellulose, or conventional cotton treated with nitric acid. By dissolving nitrocellulose in acetone, alcohol or ester, collodion is obtained. If the filter is made directly from such a solution, the pore diameter will be 700-800 millimicrons. To make filters of greater or lesser porosity, additional solvents are introduced, such as water, methyl alcohol, acetic acid, or cellosolve. Water has the ability to increase the porosity of collodion, methyl alcohol, acetic acid, and cellosolve (ethylene glycol monoethyl ether) decrease the size of the pores. For virusological work there is no necessity to increase the sizes of the pores beyond 800 millimicrons, since through such pores most of the viruses will pass. Methyl alcohol, in the amount from 1 to 15 percent, makes it possible to decrease the sizes of the pores from 800 to 100 millimicrons. To obtain pores of a still smaller size, acetic acid or cellosolve is used. Both these solvents diminish the size of the pores from 100 to 10 millimicrons.

The filters are prepared in a special chamber, consisting of a polished round glass plate with a superposed glass ring to prevent the run-off of the collodion. The glass chamber on the regulating table is placed into a container, where a constant temperature and humidity are maintained. Collodion is poured into the chamber and left there for $1\frac{1}{2}$ - 2 hours, so that the additional solvents are evaporated, with the formation of pores. To stop the evaporation, the collodion plate is submerged in water for 24 hours. With the aid of a blanking die,

RESTRICTED

RESTRICTED

plates of requisite sizes (usually 35 millimeters) are prepared. These are washed over a period of 5 - 7 days in running water, to remove the solvent. The thickness of the finished filters is 0.15 - 0.18 millimeters.

The size of the pores is determined by filtering through them of colloidal pigments of known sizes, or with more precision, by indirect data, which includes the thickness of the filter, the percent of its moisture content, and the rate of the water flow through the filter. The filters are kept in sterile distilled water (Figures 14, 15, 16).

Prior to use, the filters are sterilized by boiling for 20 minutes in distilled water.

The mounting of the filters into the Zeyts installation is conducted as follows: first, a thin rubber ring (made from glove rubber) is placed, then filter paper is placed onto the supporting netting, then comes the filter, filter paper on top, and a second rubber ring. The filter paper on top is for the purpose of detaining coarse particles, and the filter paper below is for insulation from the netting, which may damage the very thin filter diaphragm. The two rubber rings (gaskets) prevent the filter from being ruptured by the tightening of the screws. Unconditionally, all parts must be preliminarily sterilized, and the entire operation of mounting, in order to maintain complete sterility, must be done under exposure to ultraviolet rays.

Filtration is effected under negative or positive pressure. Variations in pressure must be gradual, in order to

RESTRICTED

RESTRICTED

avoid obstruction, or the simultaneous rush toward the pores of excessive numbers of particles, which may stop up the pores. In passing through the filter, there takes place the adsorption of virus particles, and the pore openings diminish in size. The coefficient of adsorption, depending on the size of the pores, varies from 0.5 to 0.35. Thus, for example, the virus of grippe, 80 millimicrons in size, will not pass through a filter with 150 millimicron pores.

Filtration is more effective, when the virus is in a bouillon medium, and not in a physiological solution medium, since the bouillon is a capillary active substance. An alkaline medium, is also favorable to effective filtration. Prior to filtration, it is necessary to pass first the liquid, in which the virus is suspended. The first 2 - 3 cubic centimeters of the filtrate is discarded, since, due to adsorption, it may not contain the virus. The total amount of the liquid to be filtered, with the diaphragm size of 35 millimeters, shall not exceed 10 cubic centimeters. The ultra-filtration method is used for sterilization, determination of virus sizes, and virus concentration.

6. The Study of Other Virus Characteristics

In studying the characteristics of viruses, their stability to physical and chemical influences is tested. This characterizes the ability of viruses to survive in an outside medium, their reaction to various temperatures and disinfectants.

RESTRICTED

To determine the effect of temperature, a virus suspension is prepared and poured into test tubes, which are then observed under various temperatures, such as room temperature (15 - 18 degrees Centigrade), and thermostat temperature (36 - 37 degrees Centigrade). To determine the effect of higher temperatures, the test tubes with the virus suspension are placed in a water bath at 50, 60 and 75 degrees Centigrade. All viruses are inactivated at a temperature above 100 degrees Centigrade (at a less high temperature they manifest various degrees of viability).

All viruses are stable to low temperatures. At minus 70 degrees Centigrade, virus suspensions can survive for years.

Exposure to the concentrated rays of the sun, as well as to ultraviolet rays, has an inactivating effect on the viruses. Diffused sunlight has a less active effect. However, if small amounts (volumetrically, 1 : 25000 in dilution) of methylene blue are added to a virus suspension, which is exposed to diffused sunlight, the virus in it will become inactivated after 15, 10, or even 5 minutes of exposure to the light. Methylene blue, added to the virus suspension, in the same concentration, but in darkness, will have no effect on the virus. This phenomenon is called the photodynamic effect of methylene blue on the virus. Under the photodynamic effect of methylene blue, the viruses of rabies, herpetic encephalitis, spring-summer, Japanese encephalitis, and others, are inactivated.

Exposed to the effect of alcohol, ester, acetone, the

RESTRICTED

RESTRICTED

viruses become inactivated in various degrees, dependent on the concentration of the above substances and the time of exposure to their effect. Some viruses (poliomyelitis, rabies) are comparatively stable to ester. Others (grippe, ectromelia) are inactivated by ester more rapidly. Various acid and alkali tests show that most of the viruses are destroyed rapidly in both acid and alkaline solutions. Various chemicals, such as phenol, formalin, potassium permanganate, and others, inactivate the viruses. However, in a 0.5 percent phenol solution some viruses retain their viability, as, for instance, the virus of rabies will, in such a solution, survive up to 3 months. Viruses, inactivated by formalin, remain immunogenic, which made the utilization of formalin in the preparation of vaccines possible.

Almost all viruses perish under the effect of lysol. A 3 - 5 percent lysol solution destroys the viruses of encephalitis, grippe, ectromelia within 3 - 5 minutes. This solution is used continuously in virusological laboratories as a disinfectant. Soap and soap solutions are also good inactivators for the viruses of the encephalitises and grippe.

The testing of the effect of various chemicals is conducted as follows. The initial virus suspension (usually a 10 percent) is mixed in test tubes with some chemical reagents, taken in a dilution that is not toxic to the test animals. After various time intervals of contact of the chemical with the virus, the contamination of the animals is effected.

- 52 -

RESTRICTED

Animals, which are stricken after the corresponding incubation periods, attest to the viability of the viruses in the mixtures. If clinically pronounced symptoms are not manifested in the inoculated animals, two additional passages are made, and only the absence of clinical symptoms in the third passage can be considered as proof of the virus inactivation.

Alongside with the ascertaining of the above cited virus characteristics, i.e. their inoculability from animal to animal by a series of consecutive passages, filterability, pathogenic characteristics, it is also necessary to study their antigenic characteristics, their morphology, conduct a histological examination of the tissues of contaminated animals, and also to investigate the dissemination of the virus in the organisms of inceptible animals. To accomplish the latter, all the organs and tissues of the contaminated animals are examined at various time intervals after inoculation, and, without fail, during the climax of the infection.

In studying the propagation of the virus of poliomyelitis, its rigid neurotropism was established. Not once was this virus discovered in the blood of the contaminated animals, while at the same time abounding in the central nervous system. Other neuroviruses are not that rigidly neurotropic. For instance, the virus of spring-summer encephalitis, after 2 minutes upon peripheral inoculation, penetrates into the blood and into the brain. In a stricken animal, the virus is found in great concentrations in the brain, in smaller con-

RESTRICTED

centrations in the blood, and other organs. In the case of grippe, the virus in the test animals is usually found in the lungs and in the upper respiratory passages. ✓

7. Serological Reactions.

As was already mentioned, there are in the blood of convalescents from a virus infection, specific virus counteracting antibodies. These antibodies are discovered by the same general methods that are used in bacteriological procedure: the agglutination test, the complement fixation test, the neutralization test. Each of these tests is described separately below.

A. The agglutination test. The first one to obtain an agglutination of virus particles was Pashen, when he combined the suspension of the elementary corpuscles of a virus vaccine with immunization sera. The suspension of the elementary corpuscles was combined with an immune serum, and the reaction was studied under a microscope. The titration standard for the agglutinins was, in some cases, up to 1:100. Such agglutination reactions were obtained with the viruses of Psittacosis and ectromelia.

The ability of the viruses to become adsorbed on colloidal particles and on bacteria, made the wider use of the agglutination test possible also with relation to other smaller viruses. Roberts and Jones (1940) described the adsorption of the viruses St. Louis and poliomyelitis on the heat-killed bacterial cells *Serratia marcescens*, with subsequent agglutination by a specific serum. When antibodies are present in the serum, the agglutina-

- 84 -
RESTRICTED

tion of bacterial cells, loaded with the adsorbed virus, will take place.

The method of adsorption of the virus of measles on *Fact. typhi abdominalis* was developed by Sergiyev, Demina, and Ryazantseva (1944). A suspension of microbes killed by heat (15 - 20 billions of microbe bodies in 1 cubic centimeter) was mixed with an equal volume of virus containing material (a pharynt gargle or hemolyzed blood from a measles patient), kept for 30 - 40 minutes at room temperature, and overnight in a refrigerator. The mixture was then washed twice in physiological solution, with repeated centrifuging. The derived antigen was tested for agglutination with serums from convalescents. For purposes of control, microbes treated with the blood or the pharynt gargle from healthy humans, were used. The control sera were sera prepared from the blood of [humans] who were never attacked by the disease. The reaction was read with the aid of a agglutinoscope.

It is pertinent to describe in this chapter the agglutination reaction of erythrocytes in the case of grippe. In 1941, Kherst discovered that the virus of grippe agglutinates the erythrocytes of a chick, and that this reaction may be arrested by the addition of an anti-grippe serum. This reaction became known as the reaction of hemagglutination, and the phenomenon whereby the agglutination of the immune serum is arrested - the reaction of arresting hemagglutination. It was subsequently discovered that the agglutination of erythrocytes may occur not only under the effect of the virus of grippe, but also under the effect of the viruses of smallpox, chicken pseudo-plague, mice ectromelia, and infectious parotitis. The

reaction of hemagglutination found wide use in the diagnostics of the grippe, with relation to both, the discovery of the virus as well as the antibodies.

The flowsheet of the original procedure in its latest version is as follows: The virus suspension of doubly increased degrees of dilution, in a dosage of 1 cubic centimeter, are made ready in test tubes, 7 centimeters high, with 0.8 centimeter inside diameter. One cubic centimeter of 1.5 percents suspension of washed chick erythrocytes is added into each test tube. The liquids are mixed, the test tubes are left for 75 minutes at room temperature. Results are judged by the visible density in the little column of the erythrocytes, which did not settle to the bottom, since the agglutinated erythrocytes will settle more rapidly.

At first, Kherst was using the visual method of comparison, as between the above test tubes and tubes containing erythrocyte suspensions of various degrees of concentration subsequently, the density of the agglutinated erythrocytes was determined with the aid of a specially designed photoelectric densitometer. The number of hemagglutinins in 1 cubic centimeter of a virus suspension, in which 50 percent of the erythrocytes are settled at the bottom after 75 minutes, is accepted as a unit.

The reaction of arresting hemagglutination is conducted as follows: 0.5 of a cubic centimeter of a doubly increased degree of dilution inactivated test serum is mixed with 0.5 of a cubic centimeter of a virus suspension, containing 4 units of hemagglutinins; to this mixture is then added 1 cubic centi-

RESTRICTED

meter of a 1.5 percent erythrocyte suspension, at the time. Results are recorded every 75 minutes. The titration standard of the serum is considered a dilution of it, in the presence of which 50 percent of erythrocytes will settle to the bottom in 75 minutes. The main condition for the precision of results in this reaction, is the rigid maintenance of the indicated interval between the moment of adding the erythrocytes and the moment of recording results.

Selov'yev and Shubladze suggested that the entire reaction be staged on a microscope slide. A drop of virus containing material is thoroughly mixed with a drop of a 2 percent chick erythrocyte suspension, on a sterile and degreased glass. After 2 - 3 minutes, the agglutination may be observed under a magnifying glass or even with the naked eye. Once the agglutination of the erythrocytes is ascertained, specific reactions with immune sera are tried. In this case, a drop of virus containing material, a drop of a 2 percent erythrocyte suspension, and a drop of a 1:10 dilution of [immune] serum are placed on the glass. Upon mixing, the reaction is observed at room temperature. When agglutination is arrested completely, as compared to the control reaction, where, in place of the immune serum, normal serum in 1:10 dilution is added, the result is recorded as positive. The drop reaction on glass is rapid and convenient, but less sensitive, as compared with the test tube reaction. The authors suggest the use of the drop reaction on glass, during epidemic outbreaks, for diagnostic examinations of the gargles of grippe stricken patients, and for the selection of the grippe-suspected gargles

- 87 -

RESTRICTED

RESTRICTED

for the subsequent virus precipitation in animals.

The same authors discovered that the virus of gripe agglutinates not only erythrocytes of chickens, but also erythrocytes of other animals and humans. The degree of the agglutination of erythrocytes of various animals is presented in Table 5.

Table 5

Erythrocytes	Degree of intensity of agglutination	Erythrocytes	Degree of intensity of agglutination
Chicks and Chickens	+++	Horses	-
Guinea Pigs	+++	Rams	-
Dogs	+++	Foxes	-
Humans	+++	Cats	-
White rats	++	Rabbits	-
White mice	++	Frogs	-

Designations: +++ (positive reaction), ++ (weakly positive reaction), - (negative reaction)

The modification of the hemagglutination reaction, suggested by Nartsisson, consists of carrying the reaction from the test tubes to a special plexiglas board with concavities equal to the bottom of the bacteriological test tube (Figure 18). It is very convenient to conduct the titration of the virus and sera in such a device, since the reactions can be recorded and their intensities compared simultaneously.

RESTRICTED

RESTRICTED

Figure 1B. Plexiglas board used for the erythrocyte agglutination test.

The titration of the grippe virus by this method is conducted as follows: into each concavity of the board, 0.2 of a cubic centimeter of a physiological solution is poured, then into the first concavity 0.2 of a cubic centimeter of the virus suspension is added, thoroughly mixed, and then, in sequence, 0.2 cubic centimeters of the mixture is carried from one concavity into the other. Thus, the coefficient of dilution equals 2. Then, to each virus dilution is added 0.2 cubic centimeters of a 2 percent chicken erythrocyte suspension, which is mixed with the virus. The board is placed for one hour in a thermostat at 37 degrees Centigrade. The control mixture is watched for spontaneous agglutination, where the erythrocytes are added not to the virus suspension, but to a physiological solution. Where agglutination occurred, the precipitation of erythrocytes is star shaped, with cusp-like edges. Where there is no agglutination, the erythrocytes settle by gravity, forming a uniform disk on the bottom. The ultimate titration standard of the virus is the maximum degree of dilution, at which there is still agglutination.

In order to determine the grippe antibodies, 0.1 cubic centimeter of a stable virus dosage of a four-fold titration standard is added to 0.1 cubic centimeter of various serum dilutions (the dilutions are prepared in the same manner as the virus dilutions). This means that, if the virus titra-

RESTRICTED

RESTRICTED

tion standard is 1:640, it is taken, for the test, in a dilution 1:160. The virus and the serum are mixed thoroughly, a 2 percent erythrocyte suspension in 0.2 cubic centimeter doses is added, and the mixture placed in a thermostat for one hour. The reaction is read in the same manner, as the titration of the virus, with the only difference that the titration standard of the serum is considered to be the maximum degree of dilution, at which agglutination is not yet occurring (Figure 19 - 20)

Figure 19 - 20. The agglutination of the chicken erythrocytes by the virus of gripe.

A- titration of virus; I, II, III - intensive agglutination, designated + + + + , + + + , + + ; IV - weak agglutination, designated + ; V - absence of agglutination, designated - ;
B- the reaction of arresting hemagglutination; I - arresting of agglutination by immune anti-gripe serum.

B. The complement fixation test. The reaction of complement fixation is becoming ever more established procedure in the diagnostics of virus diseases, complementing and widening the methods of serological analysis. This test is described in application to the viruses of vaccinia, smallpox, Psittacosis, venereal lymphogranuloma, hoof and mouth disease, rabies, gripe, lymphocytic choriomeningitis, horse encephalomyelitis and human encephalitis. The difficulty in conducting the complement fixation test, is the circumstance that a pure antigen is frequently not available. An antigen is a tissue

RESTRICTED

extract containing not only the virus, but also the ballast substances affecting the reaction progress. Particular difficulties are encountered with neurovirous antigens, since cerebral suspensions have strong anticomplement characteristics. Inasmuch as the antigens, in addition to the virus, contain other substances, capable of complement fixation, additional controls are introduced into the reaction by bringing in antigens from normal tissue of the same type. The test requires precision, concentration, and painstaking preparation of each detail.

Test tubes, pipettes, flasks must be made from neutral glass, thoroughly washed, transparent and dry. It is most convenient to use test tubes with a diameter of 1 centimeter and a height of 8 - 10 centimeters. The pipette must be precision graduated and calibrated. The physiological solution must be of precise concentration and of neutral reaction, and is to be prepared not before 24 - 48 hours prior to the test. The physiological solution may be prepared on running water, in place of distilled water, but, in that case, the water must be boiled and autoclaved. This is based on the fact that single-run distilled water usually has an acid reaction. (pH = 6.4 - 5.5), while sink water, due to its mineral salt content, has the characteristics of a buffer solution, and does not easily change its reaction. Below, the preparation of the ingredient for the test is described.

Antigen. The difficulties of working with virus antigens, particularly with antigens prepared from cerebral tissues,

RESTRICTED

was already mentioned before. Antigens from other tissues do not possess such pronounced anticomplement characteristics, and do not require any special treatment. Thus for instance, the virus of gripe is used in the form of a 5 percent lung suspension, taken from white mice, and well frozen at minus 4 - 6 degrees Centigrade, and centrifuged for 15 minutes at 2000 RPM.

For the preparation of antigens for cerebral tissue, in accordance with Cassals, a 10 percent cerebral suspension, extracted under aseptic conditions, from animals stricken with the respective virus, during the climax of the infection, is used. The suspension is prepared in physiological solution containing a 2 percent normal inactivated guinea pig serum. It is placed for 20 hours in a refrigerator at 4 degrees Centigrade, then centrifuged in a horizontal centrifuge at 2500 RPM for 30 minutes. The upper layer of the liquid is drawn off into lusteroid tubes and subjected to five-fold refrigeration (in a mixture of dry carbon dioxide with alcohol, which produces a temperature of minus 72 degrees Centigrade), then thawing out at 37 degrees Centigrade, at the time of which a flocculate appears. Finally, it is again centrifuged for one hour at 3500 RPM. After all this, the antigen is completely transparent, with a slight opalescence. A conservant merthiolate 1:10000 is poured into ampules and stored, prior to use, in a refrigerator at 4 degrees Centigrade or in a frozen state. It remains well preserved for 2 months, after which it begins to manifest anticomplement characteristics.

RESTRICTED

One difficulty entailed in the use of such antigens is that they are infectious, making their application precarious outside of special laboratories. Recently it was proposed to use an ultraviolet inactivated antigen, which is only to an insignificant degree less active than an infectious one.

Sera. Sera prepared from convalescent or immune animals are used for the complement fixation test. In order to avoid non-specific reactions with tissue antigens, it is recommended that only homologous sera are used. For instance, if the antigen for the complement fixation test is made from the cerebrum of a mouse, it is necessary to prepare the immune serum on mice, immunizing it with the mouse antigen. If the sera from convalescents are tested in the reaction, the rules of homology, as between the antigen and the serum, may not be observed. It is best to avoid the use of rabbit sera, since they frequently manifest non-specific reactions.

Prior to the test, the sera are inactivated in a water bath for 20 minutes at a temperature, which is determined for each species of animal: 56 degrees Centigrade for the guinea pig serum, 60 degrees for mouse and human sera, 65 degrees for rabbit and dog sera.

In heating over 60 degrees Centigrade, the sera must be preliminarily diluted by a physiological solution in a ratio 1:2 or 1:3, to avoid the coagulation of albumen. Under conditions of long storage, the sera acquire anti-complement characteristics.

RESTRICTED

Complement. As the complement, a fresh serum from guinea pigs, is used. A preserved complement, which over a period of 1 - 2 months does not change its titration standard, may be used. As the conservant agent (the Ginsburg and Kalinin method), a mixture, containing 0.05 gram of sodium sulfate and 0.04 gram of crystalline boric acid per 1 cubic centimeter of the serum, is used.

Hemolytic serum - is obtained by the immunization of rabbits with male goat erythrocytes. Nartsissov proposed the following diagram for rapid immunization. Into the auricular vein of a rabbit's ear, 1 cubic centimeter of washed off erythrocytes of a ram is inoculated 3 - 4 times (every other day). The serum can be taken on the seventh day, if its titration standard has reached 1:1500 - 1:3000. A serum with a three-fold titration standard is taken for the test. It is preserved in cold storage for which purpose to every 3 cubic centimeters of serum, one cubic centimeter of a solution of the following [coulometric] composition is added: 1 cubic centimeter of phenol, 4 cubic centimeters of glycerin, 20 cubic centimeters of distilled water.

Erythrocytes. Defibrinated blood of a ram is preserved in formalin, 0.1 cubic centimeter of formalin to each 80 cubic centimeters of blood, and stored in a refrigerator. The erythrocytes of the ram are washed off twice in a surplus amount of physiological solution, and suspended as a 3 percent suspension of the precipitate.

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A hemolytic system is a mixture of hemolytic serum in a threefold titration with an equal amount of a 3 percent suspension of the erythrocytes of a ram. The mixture is prepared extempore, 15 minutes prior to the test.

Reaction procedure. The first stage is the titration of the complement working dose. Complement dilutions 1:10, 1:15, 1:20, 1:25, 1:30, 1:40, 1:60, 1:80 are made ready in auxiliary tubes.

From each auxiliary tube, the complement in its respective dilution and in quantities of 0.2 cubic centimeter, is carried into the tubes of the basic row, with the addition into the same tubes of the physiological solution and the hemolytic system.

	Complement dilutions							
Ingredients	1:10	1:15	1:20	1:25	1:30	1:40	1:60	1:80
Complement in cm ³	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Physiological solution in cm ³	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Hemolytic system in cm ³	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4

The tubes are then shaken up vigorously and placed in a thermostat at 37 degrees Centigrade for 30 minutes, with a repeated shaking after 15 minutes standing in the thermostat. The working dose of the complement is the dilution in the tube, which is to the left (after skipping one) of the tube, in which the hemolysis is complete. Let us assume that this working dose is the 1:30 dilution.

RESTRICTED

The next stage is the determination of the working dose of the complement, in the presence of all the antigens and sera, which will take part in the reaction.

In this procedure, the complement dose for the main test is titrated, since the antigens and sera may in some cases cause the fixation of the complement. Again, complement dilutions corresponding to its quantities in each tube are made ready in an auxiliary row of tubes - 0.06; 0.08 cubic centimeters, and so on. The initial concentration is the complement dilution, which corresponds to the working dose, in our example 1:30.

Complement dose in cm^3	0.06	0.08	0.1	0.12	0.14	0.16	0.18	0.2
Ingredients								
Complement in cm^3	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0
(Working dose)								
Physiological solution in cm^3	1.4	1.2	1.0	0.8	0.6	0.4	0.2	-

The titration of the complement working dose, in the presence of the reaction ingredients is represented below.

Complement dose in cm^3	0.06	0.08	0.1	0.12	0.14	0.16	0.18	0.2
Ingredients								
Antigens or sera in cubic centimeters	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Complement in cm^3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Physiological solution in cm^3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

RESTRICTED

The mixtures are shaken and placed for an hour in a thermostat, after which 0.4 cubic centimeters of the hemolytic system is added into each tube. After 30 minutes, the reaction is read. The working dose is considered to be the one that corresponds to the tube, in which the hemolysis is complete. Only after this is accomplished, the main test begins.

The following ingredients enter the main test: test and control antigen, test and control sera, the complement, and the hemolytic system. Cerebral antigens are used undiluted. If a serum is titrated, it is tested in various degrees of dilution; if an antigen is titrated, an undiluted serum and antigens in various degrees of dilution are used. The complement is used in 2 - 3 increasing dosages. If full hemolysis in the titration of a working dose of the complement corresponded to dose of 0.1 of a cubic centimeter, then 1.5 of a dose will correspond to 0.15 of a cubic centimeter, and 2 doses will correspond to 0.2 of a cubic centimeter, all this with relation to the initial 1:30 dilution.

The antigens, the sera, and the complement are poured into tubes, in doses of 0.2 cubic centimeters, respectively, shaken well, and kept a temperature from zero to 2 degrees Centigrade for 18 hours after which 0.4 cubic centimeters of the hemolytic system is added into each tube, and the tubes placed in a thermostat for 45-60 minutes. Then, the reaction is read; the control tubes must show complete hemolysis, or only a slight degree of retardation, which is taken note of, when reaction is read. If

a reaction between the antigen and the serum took place, the formed compound will cause the fixation of the complement, and there will be no hemolysis. The intensity of hemolysis retardation is designated conditionally by plus signs from (+) to (+++).

With the aid of the complement fixation test, it became possible to trace the presence of a virus in the organism of an animal, in the absence of clinical symptoms. This method was suggested by Ioffe, Rozentol', and Nemzer for the diagnosis of measles. If live antigen is inoculated into an animal, it either propagates in the organism, with its quantity increasing, or degenerates, with its quantity diminishing. This can be detected with the aid of the complement fixation test, by using sera, known a priori to be immune, and virus inoculated tissue from an animal, the virus having been used as an antigen. The above authors, by using this method, determined that the virus of measles propagates in the lungs of mice, although the mice, to all appearances, remain normal.

Neutralization Test.

The neutralization test is used very widely in virus examinations. The basis for the application of this test is the fact that the viruses, in mixtures with specific immune sera, are inactivated, and their pathogenic characteristics are neutralized by the effect of the serum. The mechanism of the reaction of neutralization is not clear. Some believe

that there is a phenomenon analogous to the effect of antitoxin upon toxin, when there is a direct interaction between the virus and the serum antibodies. Others consider this reaction as a blockade of the susceptible cells by the antibodies of the serum. It is possible that the mechanism of the reaction is different in the case of different viruses. It has been ascertained that some viruses, from their neutral mixture with immune serum, can be reactivated through simple dilution by a physiological solution, by high-speed centrifuging, or filtration. The virus of grippe can be precipitated from the neutral mixture by adsorption and filtration (Smorodintsev). On the other hand, Turevich and Yanusovich discovered the complete inactivation of the virus of rabies by the serum.

Two components take part in the reaction of neutralization: the serum and the virus. Volumetrically equal quantities of non-diluted serum are combined with increasingly diluted solutions of the virus, or a constant dosage of the virus with various dilutions of the serum. The first variant is more convenient, since there is no need for the preliminary determination of the precise dosage of the virus.

The test serum is dosed out with dry sterile pipettes into sterile test tubes, in quantities required for the contamination of the animals. The number of test tubes for each serum must equal the number of virus dilutions. For purposes of control, a series of tubes containing normal serum from an animal of the same species, or from a human, and a series of tubes containing a physiological solution, are made ready.

Then, into the tubes with the sera and with the physiological solution, a volumetrically equal dose of the virus suspension in various dilutions, is added. The last dilution must correspond, approximately, to the ultimate infection dosage, and for the control test one more dilution is taken.

A separate dry sterile pipette is to be used for each separate dilution in carrying it in appropriate quantities into the tubes containing the sera, beginning with the highest dilution of the virus. The mixture is well shaken and placed in a thermostat at 37 degrees Centigrade for 1 or 2 hours. Susceptible animals are then inoculated with the mixture. In so doing, the tropism of the virus is to be taken into account: in pneumotropic infections, the mixture is inoculated through the nose, in neurotropic infections intracerebrally, in dermotropic infections onto the scarified skin.

The mixture is also inoculated subcutaneously, intra-abdominally, or intravenously, if by these methods the infection can be effected.

The quantity of the inoculation substance is determined with relation to the place of inoculation, it being the case that in all dilutions of the experimental, as well as the control, series of tubes, it must be the same.

For each series of sera, the injector must be thoroughly sterilized. The control series of tubes are used last.

The count of results is done by the number of stricken or

NOTED

perished animals over a period of time, which is no less than 2 - 3 times in excess of the usual incubation period.

The evaluation of results of the neutralization test is a very complex and responsible task. As yet, there is no adequately correct and reliable method of statistical follow-up. The computation method for the so-called immune sera neutralization index, suggested by Reed and Mench, is defective to a considerable degree, and may be used only with great caution. The results of the test are usually considered positive, if the number of mice surviving the basic test, is equal to 50 percent of the number of mice, which perished in the control test, and sharply positive, when the number of mice surviving the basic test, is in excess of 75 percent of the mice, which perished in the control test (Table 6).

Table 6. Record of neutralization test.

Sera	Virus dilution					Virus titration standard in the presence of serum	Neutralization Index
	1:10	1:100	1:1000	1:10000	1:100000		
Immune Serum No 1	+	+	○	○	○	1:16	3/25
Immune Serum No 2	+	+	+	+	○	1:5000	10
Control Normal Serum	+	+	+	+	+	1:58000	
Physiological solution	+	+	+	+	+	1:58000	

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Designations: + stricken mice; 0 non-stricken mice.

In cases, when it is difficult to decide as to what caused the death of the animal, a histo-pathological examination is to be made. In particularly responsible tests, all animals, the surviving as well as the perished, are to undergo a histo-pathological examination and the results of the test are evaluated also on the basis of morphological transformation.

Since, the evaluation of results of the neutralization test, the latest procedure, particularly abroad, if the determination of the neutralization index, by the Reed and Munch method, we describe this method below.

The neutralization index is the ratio of the virus dosage mixed with an immune serum, which will cause the death of 50 percent of the mice, to the virus dosage that will cause the same lethality in mixture with a normal serum. The neutralization index is the expression of the maximum number of lethal doses that is neutralized by a given serum, as compared to the control test, for which it is always unity. As an example, we cite the computation of the neutralization indexes for the above mentioned sera No 1 and No 2.

At first, the virus titration standard, in the presence of the given serum, and in the control test, is computed. Then, the neutralization index, designated IN, is computed as follows:

$$IN = \frac{LD_{50} \text{ of the virus mixed with immune serum}}{LD_{50} \text{ of the virus mixed with normal serum}}$$

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$$\text{For Serum No 1, IN} = \frac{1}{16} : \frac{1}{58000} = \frac{58000}{16} = 3625$$

$$\text{For Serum No 2, IN} = \frac{1}{5800} : \frac{1}{58000} = \frac{58000}{5800} = 10$$

The index, up to 10, is considered negative, from 11 to 49 - doubtful, from 50 and up - positive. In our example, it is negative for Serum No 2, consequently, no antibodies for the given virus were discovered in it. For serum No 1, the index is positive.

The neutralization test can also be conducted with the chorio-allantois of a developing chicken embryo. The use of this method, in two modifications, is suggested in the case of encephalitis St Louis: in the first modification, a ready made mixture of serum and virus is inoculated into the ovum, and in the second modification, the passive defense of the chorio-allantois by the immune serum, with subsequent contamination by the virus, is reproduced. Particularly pronounced results are obtained by coloring the chorioallantois, while still alive, with methylene blue, which renders the presence of infection nuclei ^{microscopically} macroscopically visible.

Korpevskig and Iennet made a comparative study of the results of the neutralization test with the virus of horse encephalitis (Venezuela ^{strain} stem), inoculated intracerebrally into mice, and on chicken embryos. No difference in susceptibility, as between these two methods, was discovered.

Upon familiarization with the above described most

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widely used serological tests, the question naturally arises, as to which of these is the best. There can be no universal advice that would cover the case of all viruses, and, under various conditions, one or the other method is to be preferred. In clinical diagnostics, the neutralization test can serve the purposes of retrospective diagnosis only, due to the slow accumulation of antibodies in the blood of the patient, and also due to the fact that results are evaluated after 2 - 3 weeks. This test is not used for rapid diagnosing.

The neutralization test is very important in the study of epidemiology of virus infections, and the discovery of endemic centers. In individuals, who have long since recovered from spring-summer encephalitis, for instance, neutralizing antibodies are discovered tens of years after having been originally stricken.

For rapid diagnosing, the complement fixation test is more suitable, since the antibodies, which effect the fixation of the complement, appear already during the first days of the infection, and the results of the test are evaluated the next day, after the test is made.

The neutralization test must not, however, be set off in contrast to the complement fixation test. These two tests are complementary and mutually corrective to each other, and in the study of various viruses it is recommended that both of them be used.

- 164 -

RESTRICTED

RESTRICTED

In conclusion, the Table below (Table 7) will be helpful in the selection of the specific method for the serological investigation.

Comparative data are given for the agglutination, complement fixation, and neutralization tests on the viruses of grippe, smallpox, ectromelia, venereal lymphogranuloma, measles, rabies, spring-summer encephalitis, also for the hemagglutination reaction and the arresting of the latter by immune sera.

Table 7.

Antigens		Sera	Reaction of				
Virus of	Material		hemagglutination	agglutination of volume	agglutination bacteria	complement fixation	agglutination of clonant
Grippe	Lungs of Mice	Human	+	+		+	+
		Horse	+	0		+	+
		Rabbit	+	+		+	+
		Ham	+	0		+	+
		Horse	+	0		+	+
		Rat	+	0		+	+
Grippe	Allantoic fluid	Human	+	0		+	+
		Skunk	+	0		+	+
		Rat	+	0		+	+
		Horse	+	0		+	+
Smallpox vaccine	Content of pustules	Human	+	0		+	+
		Rabbit	+	0		+	+

RESTRICTED

			(1)	(2)	(3)	(4)	(5)
Ectromelia	Affected sec-	Mouse	+	0	0	+	+
in mice	tions of skin,	Chicken	+	0	0	+	+
	Liver						
Venereal	Gall	Human	0	NS	+	0	+
Lympho-	bladder	Female	0	NS	s	0	+
granulosa		goat					
Measles	Pharynx	Human	-	+	0	0	0
	gargle, or	Rabbit	-	+	0	0	0
	hemolyzed						
	blood from						
	patients						
Measles	Livers of mice	Human	-	0	+	0	0
	infected						
	with pha-						
	rynxat gargle						
	from patients						
Rabies	Cere rum	Human	-	+	+	+	0
	of stricken	Rabbit	-	+	NS	+	0
	animals	Guinea	-	+	+	+	0
		pigs					

106 (a)

			(1)	(2)	(3)	(4)	(5)
Ectromelia	Affected sec-	Mouse	+	0	0	+	+
in mice	tions of skin,	Chicken	+	0	0	+	+
	liver						
Venereal	ball	Human	0	NS	+	0	+
Lympho-	gland	Fecale	0	NS	NS	0	+
granulosa	goat	(					
Measles	Margot	Human	-	+	0	0	0
	varicella,	Kalbit	-	+	0	0	0
	hemolyzed						
	blood from						
	patients						
Measles	Lungs of mice	Human	-	0	+	0	0
	infected						
	with pha-						
	rynt varicella						
	from patients						
rabies	Cere rum	Human	-	+	+	+	0
	of chicken	Kalbit	-	+	NS	+	0
	animals	Guinea	-	+	+	+	0
	pigs						

			(1)	(2)	(3)	(4)	(5)
Spring-	late run	human	—	—	+	+	○
summer	of stricken	force	—	—	+	+	○
encephalitis	rice	ballit	—	—	+	+	○

Designations: + positive result, ± doubtful result; — negative result;
 NS non-specific reaction ○ no test was made.

RESTRICTED

Section 8. The cultivation of Viruses outside the Organism
of Susceptible Animals.

A Virus Cultivation in Tissue Cultures

The method for the cultivation of viruses in tissue cultures is very complex and calls for a perfect mastery of the technique of growing tissue explantates in vitro. The first attempts at the utilization of tissue explantates for the cultivation of viruses outside the organism of a susceptible animal, were made with the viruses of poliomyelitis, rabies and smallpox. It was demonstrated successfully that the viruses can retain their active state in tissue cultures and induce the formation of specific inclusions in the cells of the explantates (rabies, smallpox).

Proof of a quantitative increase of the virus in tissue cultures was first furnished in the case of the virus of Rouss sarcoma, and then, in the case of other viruses. Many valuable improvements were made in the methods of cultivation, and the effect of various conditions, favorable to the propagation of the virus, were studied. The wide application of this method became possible after the Heytlands, Iac and Rivers introduced considerable simplifications into its technique.

The cultivation of viruses in tissue cultures led to the solution of a series of important problems relating to the interrelation between the virus and the cell, to the locus of virus propagation, to the mechanism of immunity.

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The tissue cultures of the virus can serve for the obtaining of antigens for serological tests. Attempts were made at the preparation of some vaccines, such as anti-yellow fever and anti-smallpox vaccines.

Before attempting to describe the technique of virus cultivation in tissue cultures, we will dwell on general postulates, which are obligatory in all cases.

For the cultivation of tissues in synthetic conditions, the presence of a nutritive medium is required. This medium must have a salt composition, approximating the salt composition of the organism, a definite concentration of hydrogen ions (pH), must contain nutritive substances and factors of growth. There are several prescriptions for such salt solutions. The most frequently used ones are the Tirode and Drew solutions.

The Tirode solution contains: 8 grams of sodium chloride (NaCl), 0.2 grams of calcium chloride (CaCl_2), 0.2 grams of potassium chloride (KCl), 0.1 gram of magnesium chloride (MgCl_2), 1 gram of sodium monophosphate (NaH_2PO_4), 1 gram of sodium bicarbonate (NaHCO_3), 1 gram of glucose, 1000 cubic centimeters of bi-distilled water.

The solution is sterilized by filtration through a Zeyts filter. Since the filtration of such a quantity of liquid is time consuming, the sterilization can be effected as follows. The solution is made ready in two parts, one part containing sodium bicarbonate and glucose, the other part

RESTRICTED

RESTRICTED

- the rest of the ingredients.

The salt solutions can be autoclaved, the glucose solutions (requiring fractional sterilisation) and the sodium bicarbonate solutions (which would disintegrate in the autoclave), are passed through the filter. Before using, both parts are reunited, with the pH of the medium equal to 7.6. As an indicator, several crystals of neutral red is added, with the medium, then, assuming a slightly rose-colored hue.

The Drew solution, in contradistinction to the Tirode solution, does not contain any glucose, and its salts are in a different ratio: 9 grams of sodium chloride (NaCl), 0.02 grams of calcium chloride (CaCl_2), 0.042 grams of potassium chloride (KCl), 0.02 grams of sodium bicarbonate (NaHCO_3), 0.01 of magnesium bi-phosphate (MgHPO_4), 0.01 gram of calcium mono-phosphate [$\text{Ca}(\text{H}_2\text{PO}_4)_2$], 1000 cubic centimeters of bi-distilled water. The pH is to be 7.4.

As a nutrition substrate, embryonic extract is added. Mostly chicken embryos are used for this purpose, since, in this case, it is easier to comply with the aseptic conditions in the extraction and treatment of the embryo. A 7 - 8 day-old embryo is extracted from the shell, thoroughly titrated and diluted with a Tirode solution, until a 20 percent suspension is obtained. The eyes [of the embryo] are preliminarily removed to avoid the introduction of pigment into the culture.

In addition, the classical method of tissue cultivation calls for the presence of a solid medium, a frame, to

- 100 -

RESTRICTED

to which the tissue fastens itself and grows on. The solid medium is usually the coagulated plasma of chickens or rabbits. It is prepared as follows: In a sterile manner, through a paraffin-impregnated cannule, the blood is taken from the carotid, then 1 cubic centimeter of a 1:500 solution of heparin is added to 20 cubic centimeters of blood. The mixture is then centrifuged in paraffin-impregnated centrifuge tubes for the separation of the plasma from the regular elements. The plasma is drawn off into cold paraffin-impregnated tubes and stored in refrigerator until called for.

The classical technique of tissue cultivation is the drop method and the flask method.

The drop method. A glass chamber consisting of a thin cover glass and ground slide, with a lune in the center, is used for the cultivation of the tissue. Several drops of embryonic extract are mixed with a Tyrode solution in a small Petri cup, then, the embryonic tissue, intended for growth, and the virus are added. A drop of this thoroughly blended mixture is carried onto the cover glass, and poured over with plasma, which upon coming into contact with the embryonic extract, is immediately coagulated. The cover glass is placed on top of the slide in such a way that the culture is in the center of the lune. The edges are poured over with sterile paraffin. The culture is placed in a thermostat. Already after 24 hours, with the aid of a magnifying glass, the growth of the tissue can be detected. The transplanting is effected after 2 - 3

- 110 -

RESTRICTED

RESTRICTED

days. The contents of the chamber are extracted into a Petri cup, and a fragment of it is cut away from the growth zone. In the same manner, fragments are cut away from morphological examinations and biological control (contamination of animals to prove the presence of the virus). This method of virus cultivation is used for morphological investigations.

The flask method. Tissues are cultivated in flat round flasks having a diameter of 3 - 5 centimeters. The flask consist of a round receive 1 - 15 centimeters high, and they are equipped with a slanting spout having a diameter of 1 centimeter and a length of 3 centimeters. The spout is plugged up with cotton and covered with a rubber hood. The advantage of this method is due to the fact that tissues can be cultivated for long periods without transplanting, since the use of the flask, instead of the tiny glass chamber, makes it possible to regenerate the medium, removing the products of decomposition and allowing the free exchange of gases. One to one and a half cubic centimeters of embryonic extract, and 0.1 - 0.2 cubic centimeters of virus, containing no more than 1000 Dlm, is poured into a flask, and some finely crushed embryonic tissue is added. Most frequently, the heart or muscles are used, less frequently - the liver and spleen. Finally, 0.5 cubic centimeters of plasma is added. The culture is placed in a thermostat at 37 degrees Centigrade.

With the accumulation of exchange products, the plasma becomes diluted. The liquid plasma is drawn off, the culture is washed through with a Tirole solution, and fresh plasma is

- III -

added. Such cultures can grow without transplanting for several months. During the virus passages, the culture is extracted into a Petri cup, and fragments are cut away, for transplanting and other purposes.

Surviving tissues. In 1928, the Keytlands simplified considerably the method of virus cultivation by the use of surviving tissues. Pulverized embryonic tissue was placed into a nutritive medium. The cells retain their living activities for 12 - 13 days, and even mitosis is observed for the first 72 hours.

Into a 25 - 50 cubic centimeter Erlenmeyer retort, 3 - 4 cubic centimeters of Tirole solution, 1-2 drops of a dense suspension of embryonic tissue, and 0.5 cubic centimeters of virus (100 - 1000 Dlm), are poured. Some viruses require the presence of certain tissues. Thus, the virus of fowl plague is cultivated in the tissues of a chicken embryo, the virus of hoof and mouth disease propagates only in the embryonic tissues of guinea pigs, for the virus of smallpox, the specific variety of the tissue makes no difference. In the presence of the virus, the dying off of the tissue occurs more rapidly; between the second and third day, as soon as the cells are perished, the quantity of the virus is diminished, therefore, the passages of the virus are usually effected after 24 - 48 hours. The passage material is the liquid medium that was purified by centrifuging. The Keytland method made it possible to pass a series of viruses over a long period of time.

Thus, the virus of yellow fever has come through a

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hundred passages, the virus of grippe - through 70, and the virus of smallpox through 50 passages. By this method of cultivation, a considerable quantity of virus can be obtained.

Cultures, prepared by the Lee and Rivers method, are a suspension of fragments of embryonic tissues and some adult animal tissues in a Tirode solution, 0.1 gram of tissue to 4 - 5 cubic centimeters of solution. The virus of vaccinia is successfully cultivated in a medium from the testicles or cornea of a rabbit, the viruses of encephalitines - in a medium from the embryonic tissues of chicken or mice embryos.

The cultivation of viruses in tissue cultures is now used comparatively little, mainly, for resolving a series of theoretical problems, already mentioned above. This method is now being displaced by the cultivation of viruses in a developing chicken embryo.

Cultivation of viruses in a developing chicken embryo.

This method is coming into ever wider use. Most of the known inciters of virus diseases of humans and animals have a more or less pronounced ability for propagation in the chicken embryo, on its membranes, in the extraembryonic fluid, and in its gall bladder. This method is utilized for various research experiments, and for the obtaining of large quantities of virus material for the preparation of vaccines.

The advantage of the method of virus cultivation in fecundated chicken ova, is the absence of spontaneous and latent virus infections, known to be present in laboratory animals and known to be the cause of errors in virusological examinations.

- 113 -
RESTRICTED

RESTRICTED

It may be added to this that the closed in chamber of the egg serves as a reliable protection against contamination with bacterial flora from the outside.

In the description of this method of cultivation, we made use of both our own experience and printed sources of information, the most important of which is the recent monograph by Beveridge and Barnett.

In a fecundated ovum the division begins even as it passes through the oviduct. By the moment the egg is ready to be laid, the embryonic disk, which is a multicellular lamina flattened out on the yolk, is formed. The embryonic disk is clearly visible as a little dark spot when egg is candled. The subsequent development of the embryo is resumed when the egg is placed into favorable temperature conditions.

As a result of division, three embryonic layers are formed: the ectoderm, the mesoderm, and the endoderm, all of which subsequently form the organs and tissues of the embryo. During the same period, the separation of the body of the embryo from the extra-embryonic area occurs. A so-called amniotic sac is formed around the body surrounding it as if with a molding. It is composed of the ectoderm and mesoderm. The amniotic sac forms a fold at the head end of the embryo, while its lateral parts grow together along a center line, as a result of which the spine of the embryo is enwrapped in two membranes.

The inside membrane, with the ectodermal leaf toward the

RESTRICTED

body of the embryo, is called the amnion, the outer membrane with the ectoderm toward the outside, is called the chorion. The chorion grows rapidly, and by the tenth day surrounds the entire content of the egg. The amnion, at first, fits quite snugly to the body of the embryo, but later becomes loose, and its chamber is filled with amniotic fluid. There is, on the average, about 1 cubic centimeter of fluid between the sixth and the thirteenth day, this amount subsequently diminishing.

The allantois is evolved on the third day of incubation as a protrusion of the ventral section of the rectum. The walls of this protrusion consist of ectoderm and mesoderm. The allantois expands, penetrates between the gall bladder, amnion, and chorion, with its outer part, which is facing the chorion, growing together with its mesodermal leaf, forming the chorio-allantois. The chorioallantois at first functions as a respiratory organ. It is amply supplied with blood vessels. Through the gall funiculus, pass two main arteries forming a capillary net near the air sac. The blood flows off through three veins, two of which run alongside of the arteries, and the third one (the main allantoic vein) lies separately. This vein is clearly visible when egg is candled. In addition, the allantois performs the function of elimination, and uric acids are accumulated in its chamber. The allantois chamber is filled with fluid, to the amount of 1 cubic centimeter on the sixth day, 6 cubic centimeters on the eleventh day, 6.4 cubic centimeters on the thirteenth day, the fluid subsequently disappearing.

The embryo derives its nutriment from the yolk, and

RESTRICTED

at a later stage, the nutritive substances proceed from the albumen sac through the adjacent chorioallantois. The diagrammatic structure of the egg, on the twelfth day of the development of the embryo, is presented in Figure 21.

Figure 21. 1 - embryo; 2 - gall bladder; 3 - allantoic chamber; 4 - amniotic chamber; 5 - extraembryonic chamber; 6 - albumen sac; 7 - chorioallantoic membrane; 8 - air space.

When working with eggs, it is recommended to organize regular shipments of fertilized eggs, preferably from white chickens. Dark shell eggs are less convenient, since they are more difficult to candle. It is desirable to use eggs weighing no less than 50 grams each that are clean after being laid, without washing. Prior to incubation, the eggs are stored for a period not to exceed 10 days at a temperature of 5 - 20 degrees Centigrade. Preliminary incubation is done in a conventional incubator. The best incubators, however, are apparatus equipped with artificial air circulation and a device for the automatic turning over of the eggs. The incubation temperature is 37.3 - 38 degrees Centigrade, with a permissible fluctuation of relative humidity from 40 to 70 percent.

In order to determine the location of the embryo and its viability, the eggs are candled with the aid of a special lamp or ovoscope. A 100 Watt electric bulb placed at the bottom of a small wooden box with an oval shaped opening in the cover, is used. The candling is done in a dark enclosure. All operations (contamination and dissection) are conducted under strictly aseptic conditions.

RESTRICTED

Contamination of the chorioallantoic membrane. For contaminating the chorioallantoic membrane, eggs with 10-12 day old embryos are used. The eggs are candled and the boundaries of the air sac outlined with pencil on the shell. On the side opposite the line, along which the growing edge of the chorioallantois is joined, a 12-millimeter equilateral triangle is drawn in the area of the strongest development of the chorioallantois. The shell is drilled through along the outline of the triangle, the triangular piece of shell is carefully lifted out and separated from the shell membrane, and a drop of sterile physiological solution is deposited on the exposed membrane. Then the shell membrane is carefully cut open so as not to damage the chorioallantoic membrane lying immediately under it. The cutting of the shell membrane is to be done along its fibers running in an oblique direction around the egg. The drop of physiological solution, in running off under the shell membrane, separates it from the chorioallantoic membrane by forming an artificial air space. Through the hole over the center of the natural air sac, the air is drawn off with a squirt; a paraffin-vaseline mixture is poured over the hole. [As a result] there will occur a displacement within the egg with the chorioallantoic membrane dropping down. All these manipulations are to be done carefully in order to avoid hemorrhages which may lead to non-specific mutations. The cut in the shell membrane is widened; part of it is bent outward or cut off, thereby making an opening for contamination.

Contamination is effected with the aid of a graduated capillary pipette equipped with a rubber bulb, the pipette to be held in a

RESTRICTED

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vertical position. The contaminating substance in a quantity of 0.05 cubic centimeter is deposited on the chorioallantoic membrane. The hole in the shell is closed with a sterile cover glass which is affixed with a paraffin-vaseline mixture fed from a Pasteur pipette all around the above described triangle outline on the egg shell. This mixture is not to run over the edges of the shell. Cellophane may be used instead of cover glass and it should be prepared in a triangular piece of size somewhat larger than the opening. The cellophane triangle is first wetted in boiling water, as a result of which it becomes sterilized and more elastic. It is then immersed in a 10 percent solution of white of egg with the addition of some chloroform as an antiseptic, after which it is applied to the opening in the shell where it will stick well.

Upon contamination the egg is not to be turned to prevent disturbing the artificially created air space. The period of the [contaminative] incubation is usually 2-3 days; but depending on the characteristics of the virus being introduced it may vary.

There is a simplified method for the contamination of the chorioallantoic membrane which does not necessitate the removal of the shell membrane. With the aid of a reciprocatingly rotating disk of a dental drilling machine an oval hole 3 x 2 millimeters is made in the egg shell. This method makes it easier to avoid damaging of the shell membrane and the underlying chorioallantoic membrane. The shell area around the hole is immediately covered with a sterile melted mixture of paraffin and wax preventing the

- 118 -

RESTRICTED

RESTRICTED

mixture from running into the hole. Onto the exposed shell membrane a drop of sterile liquid paraffin is deposited in order to make it more translucent. Then it is perforated with a needle at the center of the hole in the shell and 0.05 of a cubic centimeter of contaminating substance is deposited on it. The air is pumped off with a rubber syringe through the hole in the natural air sac. As a result there is displacement in the egg and the contamination substance becomes evenly distributed along the chorioallantois. The hole is then smeared over with a paraffin-vaseline mixture.

Upon incubation the contaminated eggs are placed in a Petri cup at the bottom of which there is some cotton soaked in disinfectant solution. The hole in the shell is unsealed and the shell removed down to the level of the chorioallantois. With the aid of pincers the chorioallantois is then cut off along the boundary of the artificial air chamber in such a manner as to take in the whole area of contamination. In order to preserve the virus the unwashed membranes are carefully transferred to a Petri cup and placed for several hours in a refrigerator to remove the blood and the flow-off liquid. The substance is controlled for sterility.

If it is necessary to count the nuclei of infection the membrane is washed off in a 10 percent formalin solution in order to inactivate the virus, spread upon a glass plate, and the nuclei of infection counted under a magnifying glass.

Contamination of the amniotic chamber. To introduce the con-

RESTRICTED

RESTRICTED

mination into the amniotic chamber a mark is made on the shell while candling at the point of origin of the allantoic vein. This point is always close to the air space and can be identified either by the shadow of the most lively allantoic vein or by the place of junction of the two large veins. One of these points is fixed. The spot for the introduction of the contamination is determined by the following rule: If the egg is held with the blunt end toward oneself, the spot for contamination will lie approximately 1.5 centimeters in a clockwise direction from the sharp end on the great diameter of the egg, i.e. closer to the air space, and is rarely inaccessible. In some eggs it is not possible to determine the location of the allantoic vein, and they should be used for other contamination methods. On the marked wall of the egg an oval 2 x 1.5 centimeter hole is drilled and inside the oval two 1-centimeter cuts are made near one end so that a triangle with one bow-shaped side is formed.

To introduce the contamination into the amniotic chamber it is necessary to have the operating area illuminated with a powerful artificial light. The shell fragment forming the triangle is removed and the chorioallantoic membrane is depressed with the aid of manipulations analogous with those used in the case of chorioallantoic contamination with the exception that the use of the physiological solution is not obligatory. The remainder of shell inside the oval is removed and the underlying shell membrane is cut off. The embryo usually lies directly under the hole. The section of the chorioallantois containing few blood vessels is pressed together with curved pincers and a cut 0.5

- 120 -

RESTRICTED

RESTRICTED

centimeter long is made carefully, avoiding the penetration of the amnion into the chorioallantoic fold.

A Pasteur pipette with a beveled end is used for the inoculation. First 0.05-0.25 cubic centimeter of the contamination substance, then 0.05 cubic centimeter of air, is collected with the aid of a rubber syringe. The amnion is grasped at the place of the cut in the chorioallantois and is carefully perforated with the pipette. First the air from the pipette is forced in and the formed bubble indicates that the inoculation spot has been selected correctly. Then the rest of the substance from the pipette is introduced. The hole in the shell is covered with a cover glass, which is fastened onto the shell with a paraffin-vaseline mixture. By using this method of sealing, a constant check as to whether the embryo is still alive can be kept and the amniotic fluid for examinations required from time to time is within easy access. The cover glass is easily removed by singeing its surface in the flame of a burner. The incubation period depends on the virus but usually does not exceed 3-5 days. The embryo is observed daily. The embryos that perish within the first 24 hours upon inoculation are excluded; the ones that perish after that are examined.

By a simplified procedure the contamination is effected with a needle syringe through a small hole in the egg shell, while the egg is candled with the aid of an ovoscope. The point of the needle is guided into the embryo. The correct position of the needle is determined by the fact that the embryo moves with the motion of the needle. After this is established the contamination substance is forced in.

RESTRICTED

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By contamination into the amniotic chamber, the virus is given the opportunity to penetrate into and propagate in a variety of cells which are in direct contact with the amniotic fluid. This method turned out to be very valuable in the case of the virus of grippe, since it facilitates the invasion of the lungs of the embryo where the virus is propagated. In 13-14 day old embryos the parabronchial epithelium begins to gemmulate and form pulmonary air capillaries, thereby increasing the number of specialized respiratory cells. At the same time the mobility of the embryo is increased with the result that the amniotic fluid moves to and fro along the trachea carrying the infection into the lungs.

In determining the mutations upon the contamination of the embryo particular attention is given to the quantity and characteristics of the amniotic fluid, examination of the lungs, and at times the tracheal fluid. This fluid, more or less turbid in contaminated eggs, is either perfectly clear or contains individual turbid flakes in normal eggs. A drop or two of the liquid obtained is collected into a capillary tube with a soldered end. The fluid in this tube is centrifuged and from the precipitate a smear is prepared on a microscope slide. The smear is colored by the Romanovsky-Giemsa, Leishman, or other method. Then the typical damages to the cells can be discovered under the microscope.

Upon extracting the tracheal fluid the body of the embryo is dissected and the lungs extracted. In normal embryos the lungs are of a pale rose color up to the 15th day of incubation, becoming subsequently dark red. The lungs of embryos stricken by the virus

RESTRICTED

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of grippe have the same color. The amniotic fluid is clear and its volume fluctuates within the norm of 0.5 to 1 cubic centimeter.

With the grippe infection the amount of this fluid decreases until it disappears completely.

Micro sections of lungs colored with hematoxylin-eosin show the characteristic changes occurring during grippe, psittacosis, and at times fowl plague.

Contamination of the Yolk. For contamination into the yolk membrane, eggs that have passed through 5-6 days of incubation are used. Upon candling, an incision is made in the center of the egg. The shell in the area of the incision is first smeared with an iodine solution. The contamination is effected with a conventional syringe equipped with a Number 20 or Number 21 needle which is guided through the incision into the center of the egg to a distance of about 3 centimeters, after which the hole in the shell is closed up by a paraffin-vaseline mixture. The contaminative material is introduced in quantities from 0.25 to 1 cubic centimeter. When this method is used, the presence of infection is determined either through the mortality of the embryos or by microscopic examinations of smears.

A simple method for the extraction of the yolk from a contaminated embryo without the risk of pollution is to suck the yolk out through the needle immersed in it with the aid of the syringe. If the needle is in the yolk to a distance not exceeding 1.5 centimeters and the egg is lying with its air sac upward, one cubic centimeter or more of fluid can be collected. If it is necessary

RESTRICTED

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to collect the entire yolk and the yolk membrane the shell and chorioallantoic membranes are dissected and the yolk membrane is extracted into a petri cup.

Contamination of the Allantoic chamber. To introduce the contamination into the allantoic chamber, 10 or 11-day incubated eggs are used. In cradling the spot is marked where the chorioallantois is at its maximum development but with a minimum of large blood vessels. The shell is notched to a length of 3 millimeters and width of 1 millimeter without touching the shell membrane. Contamination can be effected by making a perforation with the syringe needle, introducing it through the shell membrane and the underlying chorioallantois to a distance of several millimeters into the depth of the egg. In using another method the egg is placed into a support with the notch upward; an area of 1 square centimeter around the notch is smeared over with a melted paraffin-wax mixture. Five hundredths cubic centimeter of the contaminative substance is taken into a Pasteur pipette; part of it is dropped over the notch, then a perforation is made with the needle through the drop of underlying paraffin-wax mixture and membrane to a depth of several millimeters. The needle is then taken out and as soon as the fluid runs off into the perforation made by the needle, the remaining substance is let out from the pipette. The hole is sealed with a melted mixture of paraffin and wax. This method is most frequently used for the cultivation of the virus of grippé. In cultivating this virus, the eggs upon contamination are incubated for 42-48 hours, then cooled in the refrigerator for 2-16 hours. To collect the allantoic fluid, the shell over the air sac is

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cleaved and the air sac removed with the aid of pincers. In doing this the egg is held in the hand at some angle to the horizontal surface to prevent the cleaved shell from falling onto the shell membrane against which the chorioallantois is located. The egg is then put in a vertical position into the support; the shell membrane and the chorioallantois are cut through with scissors and, with the aid of pincers, folded over beyond the edges of the shell. The allantoic fluid is then drawn off.

Other methods of contamination. Methods of contaminating the embryo proper are also used although not as frequently as the above described methods. The principal ones are intra-cerebral and intra-muscular.

For intra-cerebral contamination 6-13 day-old embryos are used. The eggs are candled and dissected in the same manner as in the case of amniotic contamination. The contaminative substance is introduced with the aid of a tuberculin syringe equipped with the finest needle; jerky movements are to be avoided. As soon as the head of the embryo is accessible for the injection, a sudden pressure is applied so that the needle penetrates the cranium. From 10 to 30 percent of the embryos perish from trauma as a result of this procedure. In contaminating embryos in later stages of incubation, another method is frequently used. A hole is made through the chorioallantois; the head is held with the aid of curved out pincers through the eye socket and the contaminative substance is inoculated into the brain.

Intra-muscular contamination of the embryo is effected in

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the same manner, with the needle penetrating into the muscles of the pectoral chamber.

The methods of extraembryonic chamber, albumen sac, and intravenous contamination which did not find any practical application are not described here.

Typical changes induced by the viruses are usually most sharply pronounced in the chorioallantoic membrane. This membrane is a very thin layer of live tissue of a very simple histological structure and therefore the various pathological changes in it are frequently similar to each other. Changes evolved as a result of virus contaminations are characterized by the formation of round, opaque, inflammatory nuclei of various sizes, sometimes with a necrotic center (Figures 22, 23, 24, and 25, according to Beveridge and Barnett).

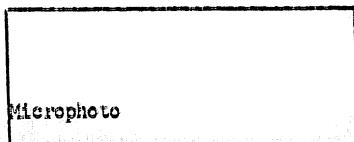


Figure 22. Changes in the chorioallantoic membrane induced by the virus of vaccinia on the third day after contamination.

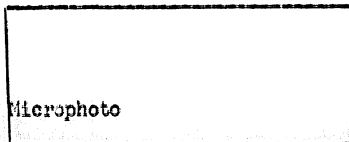


Figure 23. Changes in the chorioallantoic membrane induced by the virus of ectromelia on the third day after contamination.

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Microphoto

Figure 24. Changes in the chorionicallantoic membrane induced by the virus Louping ill. on the second day after contamination.

Microphoto

Figure 25. Changes in the chorionicallantoic membrane induced by the virus of grippe on the second day after contamination.

In studying the macroscopical changes, it is necessary to constantly bear in mind the possibility of the formation of non-specific, but similar to the above, changes in the chorionicallantoic tissue which, for example, appear as a result of trauma.

5. Microscopic Examinations

(with the consultation of R. M. Shen)

In the study of virus infections, the microscope is used with three basic ends in view: (1) searching for the elementary corpuscles of the virus; (2) searching for inclusions; and (3) the study of pathological changes in the virus stricken tissues, i.e. the study of reactive processes of the organism. In this chapter the emphasis is on the first two objects and data relating to the most popular methods of research is presented. Data on patho-histological changes due to individual virus infections is presented in the second part of the book.

Elementary corpuscles may be discovered under the micro-

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scope both in smears and imprints as well as micro sections taken from infected organs. Inclusions may be discovered in smears and imprints but a correct and clear impression is obtained only through the study of the material in histological microsections.

In preparing material for microscopic examinations it is necessary to remember, for each given virus infection, the organs that are most likely to be stricken and which sections of the stricken organs are to be treated in order to insure optimum conditions for the identification of the given infection.

The organs designated for microscopic examination are subject to fixation which is to be effected as quickly as possible following the death of the animal. It is even recommended to put the test animals to death during the period of agony. The best method is the introduction of air intravenously which eliminates esterification resulting in hemorrhages that will interfere with the proper study of the preparation. In dissection the organs are to be extracted with great care since mechanical lacerations and displacements of tissue interfere with the proper accounting of the topographical propagation of the lesions, which is of great importance in the study of the pathogenesis of an infection.

Fixative liquids. The selection of a fixative is of primary importance in the microscopy of virological infections. It is an established fact that the clarity of the image of inclusions and elementary corpuscles depends to a considerable degree on the composition of the fixative. Ethyl alcohol and formalin, which are the standard fixatives used in general pathology, cannot

RESTRICTED

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be successfully utilized in virological technique. Alcohol, being a mild reducing agent, does not penetrate well into the tissues being only a moderately fair fixative for cytoplasm and a completely ineffective fixative for the nucleoli, as a result of which the requisite contrast coloring is not successful. Formalin is a fairly good fixative for cytoplasm and an ineffective fixative for the nucleoli. However, in combination with other chemical reagents alcohol and formalin enter into the composition of the most widely approved fixatives.

(1) The Dubosk-Brasil - Euen mixture contains: 1 gram of picric acid, 150 cubic centimeters of ethyl alcohol, 60 cubic centimeters of a 40 percent formalin solution, and 15 cubic centimeters of glacial acetic acid.

This mixture is an equally effective fixative for the cytoplasm and nucleolus. The fixation is gradual and fractional, as a result of which there is almost no formation of artefacts. This mixture is an effective fixative for most of the tissues of the animal bodies with the exception of testicular tissue.

The amount of fixative used is to be in a definite volumetric ratio to the tissues involved; 30:1 is the optimum ratio. Skin and mucous membranes are fixated after thorough straightening on a cover glass or paper. For the fixation of the eye, it is recommended to introduce several drops of the fixative into the anterior eye chamber, after which it is immersed in the liquid. Lung tissue, before being immersed, is squeezed with pincers to eliminate the air. The time of fixation depends on the size of

RESTRICTED

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the fragments. For tissue fragments 2 x 3 centimeters with a thickness of 5 millimeters, effective fixation is attained in 3 - 5 days. For smaller fragments 2-3 days are sufficient. The duration of staying in the fixative has no negative effect upon the tissue. After fixation has been effected the tissue fragments are washed for 2 hours in running water.

(2) The Taenker mixture contains: 5 grams of mercuric chloride, 2.5 grams of potassium bichromate, 1 gram of sodium sulfate, and 100 cubic centimeters of distilled water. Five cubic centimeters of acetic acid is added at the moment of using the mixture.

In this fixative tissues are not to be kept over 24 hours and therefore only small thin fragments of tissue are to be treated in it. There is no point in using this fixative for larger fragments since they do not become thoroughly saturated, and a longer stay in the mixture desiccates the tissue. The fixated objects are washed in water for no less than 24 hours to eliminate the bichromate; then they are iodized (passed through 70 percent alcohol tinted with iodine until the color of weak tea is attained). The iodizing is done to eliminate the mercuric chloride (the iodine eliminates the precipitates by forming with them mercurous iodide). Iodine is then eliminated by washing in a 0.25 percent solution of sodium hyposulfite.

(3) The Khell and Maksimov mixture contains: 5 grams of mercuric chloride, 2.2 grams of potassium bichromate, and 100 cubic centimeters of distilled water. Five cubic centimeters of

RESTRICTED

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neutral non-diluted formalin and 5 cubic centimeters of a 2 percent solution of osmic acid is added to 50 cubic centimeters of the mixture just before using.

Time of fixation is 12-24 hours. Then the tissue fragments are washed in running water for 24 hours, iodized, and washed with hyposulfite.

The preparation of microsections. The most widely used are the paraffin microsections which make it possible to attain very thin slices not exceeding 3-5 microns in thickness. After fixation the pieces are washed (in a manner dependent on the type of fixative used), then passed through alcohols of rising strength; 4 hours in 70-proof alcohol, 18 hours in 96-proof alcohol, 1 hour first dewatering in absolute alcohol, and 2 hours second dewatering in absolute alcohol. The dewatered material is passed through paraffin solvents (xylene and toluene) for 3-5 hours with a one-time change of solvent after 1 hour. From the xylene, or toluene, the fragments are carried onto filter paper, slightly squeezed out, and immersed in melted paraffin for 4-5 hours. It is best to cover the fragments with paraffin on deep watch crystals since this makes it possible to so locate the fragments in the block that the requisite zone will be in its most convenient position. The watch crystal is slightly warmed over a burner; a small quantity of paraffin is poured on it, and the contemplated tissue fragment is placed in the center in such a way that the zone to be examined is toward the bottom. To obtain an adequately high block paraffin is added several times immediately upon the solidification of the preceding dose. The solidified blocks are taken off the

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glass and the surplus paraffin is trimmed off.

For a rapid histological evaluation of test substances the following fixation method suggested by Shen gave satisfactory results. Small tissue fragments with an area of 1 square centimeter and a thickness of 2 millimeters undergo fixation in a buen mixture for 1 hour (30 minutes at room temperature and 30 minutes at 56 degrees Centigrade); are passed for 30 minutes through 96-proof alcohol and twice (20 minutes each time) through xylene at 56 degrees Centigrade and then paraffinized for an hour. Blocks and sections not to exceed 4-5 millimeters in thickness are prepared. The sections are rapidly dried by repeated passing over a burner flame. Staining is done in accordance with the morphological characteristics of the given infection in order to bring out the histopathological changes, the inclusions, and the elementary corpuscles.

In accordance with the Lepin method, fixation is done in an equi-volumetric mixture of glacial acetic acid, acetone, and a saturated solution of mercuric chloride in absolute alcohol for the duration of 15 minutes at 57 degrees Centigrade. Then a double 20-30 minute pass through absolute alcohol; a 15-minute pass through toluene, and a 30-minute pass through paraffin. The entire procedure, by the first or the second method, takes 4-5 hours.

Staining methods. Staining with hematoxylin-eosin -- There are many methods for the preparation of hematoxylin. All of them are satisfactory.

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acidic hematoxylin by the Mann method: 2 grams of hematein, 10 cubic centimeters of glacial acetic acid, 100 cubic centimeters of 95-proof ethyl alcohol, 100 cubic centimeters of glycerine, 100 cubic centimeters of distilled water, 10 grams of potassium alum.

The dye is dissolved in the indicated volume of acetic acid to which 25 cubic centimeters of alcohol is added. After the dye is completely dissolved, which takes place in 24 hours, the remaining 75 cubic centimeters of alcohol and glycerine are added. The alum is dissolved in hot water and added to the dye. Upon thorough mixing, the dye is filtered and stored in the dark in effectively closed flasks.

Eosin-orange g contains: 10 cubic centimeters of a one percent water solution of eosin, 10 cubic centimeters of a one percent water solution of orange, and 80 cubic centimeters of distilled water.

Two methods of staining with hematoxylin may be used: the progressive and the regressive. In the first procedure the deparaffinized preparation is stained for 3-5 minutes in hematoxylin and differentiated for 10-20 minutes in running water; in this the preparation assumes a blue coloring. The background of the preparation undergoes an additional staining for 1-2 minutes in eosin-orange g without subsequent washing, after which the preparation is dehydrated and placed into balsam. In the ~~regressive~~ procedure the preparation is stained in hematoxylin for 20-30 minutes, differentiated in acidulous 95-proof alcohol consisting of a mixture of 15 cubic centimeters of alcohol and 5 cubic centimeters of hydrochloric acid until the appearance of a pale violet hue; the

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background is additionally stained with eosin orange P for one minute, without subsequent washing; the preparation is dehydrated and placed into balsam. These procedures are used for tentative purposes in order to determine the presence of the reactive process and the state of the nucleoli of the cells.

Staining by the Romanovsky method: There are many procedures for this method -- we will describe only two. In the first procedure the material undergoes fixation in a tannin mixture and paraffin sections are made. Deparaffinized preparations are stained at 4-5 degrees Centigrade with a freshly prepared Romanovsky-Giemsa solution in a ratio of 3 drops of dye per 2 cubic centimeters of neutral distilled water (pH = 6.9-7.3) previously boiled in an open container for 24-48 hours with two changes of the liquid. The mixing is done carefully without shaking. After being washed with distilled water the preparation assumes a dark blue color. The preparation is now dehydrated and differentiated: first in a mixture of one part of oil of cloves and 9 parts of absolute alcohol, then in pure absolute alcohol (to eliminate the remaining oil of cloves) until the preparation assumes a violet-rose hue. It is recommended that the results of differentiation be checked under the microscope. Then there is a two-time pass through toluene and the preparation is placed into balsam.

This procedure produces good results for the study of the structure of virus inclusions and elementary corpuscles and also separates effectively bacterial flora and protozoa.

In using this procedure the following conditions are to be

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observed: the glass and porcelain containers must be absolutely clean and used for this specific staining method only. After use the containers are washed with soap and running water, wiped, rinsed in distilled water and dried. The preparation is stained in a vertical position and no metal instruments are to be used in moving it from place to place.

For a rapid staining by the Romanovsky method, 1-2 drops of the stock Romanovsky dye are placed upon deparaffinized and distilled water washed microsections and, within 3 minutes, 30 drops of neutral distilled water are added and thoroughly mixed with the dye. After 25-30 minutes the preparation is washed in distilled water, decentered, differentiated and placed in alcohol.

The Romanovsky stain brings out effectively the intracellular inclusions and the elementary corpuscles but the rapid method results in less stable preparations.

In the Romanowski-Gryus procedure, the preparation undergoes fixation in a basic mixture or in one of the mercuric chloride fixatives. The mixture is prepared from 0.75 gram of a dry Romanovsky-Giemsa powdered dye, 25 cubic centimeters of glycerine, and 75 cubic centimeters of methyl alcohol. The mixture is slowly dissolved with periodic shaking in the course of 7-8 days. Such a basic solution stays well preserved. For a working solution, 10 cubic centimeters of the basic solution is mixed with 90 cubic centimeters of alcohol after which the mixture is filtered.

For staining, the deparaffinized sections are placed in a working solution of the dye taken in the following proportions:

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2 drops of dye to 2 cubic centimeters of distilled water. The staining proceeds for 21-48 hours after which the sections are washed in distilled water and are further differentiated by a mixture of one gram of orange G, 1 gram of trinin, 100 cubic centimeters of distilled water until preparation assumes an orange hue. Then the preparation is deuterated and placed into balsam.

In the triple staining, in accordance with Unna, the deparaffinized preparation is kept for 15 minutes in a 1 percent solution of orange G, washed in distilled water, and kept for 40 minutes in a 1 percent carin solution. After thorough washing in distilled water the background is colored with a solution of polychrome blueing (1:5).

The effective time of staining is 15-30 minutes for nerve tissue, liver and kidneys, and 10 minutes for lymphoid tissue. After a repeated washing in distilled water and deuterating, a differentiation is effected with the aid of oil of clover mixed with absolute alcohol in a ratio 1:9 until the appearance of a light violet hue. After eliminating the oil of clover by washing in absolute alcohol and a double pass through toluene, the preparation is encased in balsam.

This method results in an effective contrast staining; exposes the bacterial flora, protozoa, inclusions of smallpox and ectromelia, and the elementary corpuscles of venereal lymphogranuloma and psittacosis. The Negri corpuscles are not stained by this method.

Staining by the Mann method is done as follows: The de-

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paraffinized preparations are stained for 4 hours at 37 degrees Centigrade and for 18 hours at room temperature in a mixture prepared extempore, which consists of 17 cubic centimeters of a 1 percent water solution of methyl blue, 23 cubic centimeters of a 1 percent eosin solution, and 40 cubic centimeters of distilled water. In such a mixture there is no precipitation of methyl blue sediment. After washing in distilled water and dewatering, the preparation is differentiated in caustic alcohol of 3 concentrations: 30 cubic centimeters of absolute alcohol with 30 drops of a one percent solution of caustic soda in absolute alcohol (alkali); 30 cubic centimeters of absolute alcohol with 20 drops of alkali; and 30 cubic centimeters of absolute alcohol with 10 drops of alkali. Differentiation is done in Petri cups with the liquid mixed at all times for its uniform distribution in the preparation. When the preparation assumes a light rose hue, it is passed through absolute alcohol and differentiated in alcohol acid, for which purpose the preparation is placed into a jar with acidulous alcohol: 30 cubic centimeters of absolute alcohol with a drop of acetic acid added. The preparation assumes a sky-blue hue. It is then passed through absolute alcohol, xylene, or toluene and enclosed into neutral balsam.

This is the best method for the developing of oxyphile inclusions in the nucleoli as well as in the plasma; it is the standard method for the staining of the Negri and Guarnieri corpuscles.

In staining by the Turevich method, the material undergoes fixation in the Khell-Maksiman liquid, poured over into celloidin,

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then into paraffin, as per Chernyakhovskiy. Well dewatered fragments are carried from the absolute alcohol for several days into a 3 percent, and for 4 days into a 4 percent, solution of collodion in oil of cloves. From the collodion the fragments are passed consecutively through chloroform, chloroform with paraffin from 30 minutes to one hour, and twice or thrice through paraffin melted at a temperature of 56-58 degrees Centigrade. Then the micro-sections are iodized for 30 minutes in a 70-proof alcohol with several drops of iodine added and passed through a 0.25 percent hyperulfite. After washing in water the preparation is pickled for 20 minutes in a 2.5 percent solution of ammonium ferric alum and washed for 10 minutes in running water. The staining of the preparation is done in a 1:5000 solution of azure in distilled water for 24 hours. Then follows washing in water and differentiation in a water solution of picric acid, again washing in water and differentiation in a 5 percent solution of tannin. After a thorough washing in water and rapid dewatering, the preparation is passed through bergamot oil and enclosed in balsam.

For the rapid staining of Negri corpuscles Turevich suggests the following method: Brain fragments undergo fixation in acetone or in accordance with Shaudin (but not in formalin); placed in Veygert ferric hematoxylin for 2 minutes; washed in water; placed for one minute in a 1 percent water solution of acidie fuchsin; rinsed in water until the appearance of a pale rose hue, then treated with a mixture of equi-volumetric saturated water solution of picric acid and a 96-proof alcohol; rapidly washed in water; desiccated with filter paper; placed for a few seconds

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first in absolute alcohol, then in origanum oil, xylene; and enclosed in balsam.

The Negri corpuscles, even the tiniest ones, become a bright red after staining with the cells stained by hematoxylin; the nucleoli black and the background a very transparent yellow.

Examination of infectious material in impressions and smears.

Smears are prepared in the usual manner from well crushed material or by way of impressions. In order to obtain good preparations, chemically clean and degreased glass is to be used. With some experience it is possible to obtain in the impressions a monocellular layer of the material in which the tissue elements retain their normal correlations. The freshly made preparation immediately undergoes fixation. Good results are obtained by the combined use of Buan and mercuric chloride fixative. Some scientists use conventional fixatives such as equi-volumetric mixtures of absolute alcohol and ester, methyl alcohol and acetone. The preparations undergo fixation in a vertical position. The time of fixation is determined by the permeability of the fixative liquid and it fluctuates between 10 and 30 minutes.

Staining by the Murontsev method is used for rapid diagnostics of rabies and it is done by the following procedure: The freshly made non-desiccated smears undergo fixation in acetone or in the Shaudin mixture (1/3 saturated solution of mercuric chloride and 2/3 absolute alcohol), or in methyl alcohol for 30 minutes at 37 degrees Centigrade; then washed in water (and iodizing if the fixation is done in the Shaudin mixture); stained in Manson

blue for 10 minutes; washed in water; differentiated in a 5 percent tannin solution, and dewatered in a mixture of absolute alcohol and acetone.

In staining by the Mann method the fresh smears, after fixation in iodized acetone, are washed in pure acetone or 100-proof alcohol, washed for 10 minutes in running water, stained in a Mann mixture for 30 minutes at 37 degrees Centigrade, differentiated in caustic alcohol and alcohol acid (the same as used in the case of microsections) and enclosed in canada balsam.

The elementary corpuscles can be examined in smears, impressions, and pure suspensions. Pure suspensions of the elementary corpuscles are obtained from material in which they abound. For example, the smallpox virus cultivated on the chorioallantois of a chicken embryo. Ten chorioallantoic membranes taken 48 hours after the inoculation of the virus (the Farnett method) are washed twice in a Tyrode solution, each separately, after which the membranes are disintegrated with scissors and triturated in a beater containing quartz sand in 15 cubic centimeters of a buffer solution (pH = 7.2).

After centrifuging for 5 minutes at 1800 revolutions per minute the surface liquid is drawn off and preserved as Number 1. The residue is again shaken up thoroughly in 15 cubic centimeters of a buffer solution and again centrifuged at 1800 revolutions per minute. The secondary residue is discarded; the surface liquid is mixed with the Number 1 liquid; the mixture centrifuged for an hour at 10,000 revolutions per minute for the precipitation of the elementary corpuscles. The entire residue is diluted in 10 cubic

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centimeters of a buffer solution ($\text{pH} = 8$), to which one cubic centimeter of a one percent solution of trypsin is added. The dilution is left for one hour at 37 degrees Centigrade. The crystallized trypsin is first treated with ethyl ester and then petroleum ester; a one percent purified trypsin in distilled water is sterilized by filtration through Chamberland candles.

After a triple washing and centrifuging of the elementary corpuscles for 30 minutes at 3,000 revolutions per minute the residue is diluted by a buffer solution ($\text{pH} = 7.2$) and centrifuged for 10 minutes at 3,000 revolutions per minute. The residue is now discarded since the elementary corpuscles remain in the liquid over the residue in a state of suspension.

To obtain preparations the suspension of the elementary corpuscles is carried in small drops by a capillary pipette onto an absolutely clean and degreased plate (the liquid surplus is drawn off). The thin layer of suspension in the preparation is desiccated in a horizontal position in closed boxes; placed in the dark for 24 hours at 37 degrees Centigrade; then washed in distilled water for 10 minutes in a vertical position; desiccated for an hour at 37 degrees Centigrade, and again washed in distilled water prior to staining.

The Morozov method is widely used for the staining of the elementary corpuscles.

In staining by the Morozov method, three reagents are preliminarily made ready. The first reagent, the Pyre liquid, consists of 2 cubic centimeters of 40 percent formaldehyde, one cubic centi-

RESTRICTED

meter of glacial acetic acid, and 100 cubic centimeters of distilled water. The second reagent, a mordant, consists of 5 grams of tannin, 100 cubic centimeters of distilled water, and one cubic centimeter of phenol. The third solution is prepared as follows: 5 grams of silver nitrate is dissolved in 100 cubic centimeters of distilled water; then a strong (25 percent) ammonia solution is added gradually until practically the entire primary residue is dissolved (only a slight opalescence is to remain). The silver solution is stored in a dark glass flask with a ground glass stopper and it is diluted with distilled water in a ratio 1:10 before using.

Thin smears or impressions on microscope slides, dehydrated in the air, are immersed for 2-3 minutes in distilled water in a vertical vessel prior to staining. Then the first solution (the Pyre liquid) is poured over the preparation for one minute; the preparation is washed in water and the second solution (the mordant) is poured for 1-2 minutes while preheating until the appearance of vapors. The mordant is thoroughly washed away with distilled water and the third solution (the silver solution) is poured for 1-2 minutes with slight preheating until the preparation assumes a dark brown color, after which it is thoroughly washed in water and air dried. To avoid discoloration the preparation is enclosed in paraffin oil under a cover glass. The elementary corpuscles can then be seen as dark brown, almost black, small grains against the light brown background of the preparation.

The staining by the Gertsberg victoria blue method is recommended for the differentiation of the elementary corpuscles

RESTRICTED

by the degree and rapidity of fixation of a given color. As indicated by the author, the smallpox virus is stained in 15 minutes, the virus of ectromelia in 10-20 minutes and the virus of herpes in 30 minutes. A 3 percent solution of victoria blue is heated for 30 minutes in a water bath at a temperature of 60 degrees Centigrade. The solution ripens for 14 days and is stored in a dark vessel. It is filtered prior to use.

Two methods of staining are used. In the first method the preparation is stained without preliminary treatment and, after a double washing in distilled water, it is desiccated and enclosed in canada balsam. The background in this preparation is not stained; the cellular protoplasm is a light sky blue; the nucleoli are violet, and the elementary corpuscles are a bright blue. In the second method the preparation is pickled in a saturated solution of citric acid for 3 minutes. After a double washing in distilled water for 10 minutes the staining is done in a 3 percent solution of the victoria blue and acidified with citric acid (10 cubic centimeters of dye with 0.5 cubic centimeter of a saturated solution of citric acid) for 10 minutes at 37 degrees Centigrade. Then follows a double washing, desiccation and enclosing into balsam.

In staining by the Mikolau method, smears or dried drops of elementary corpuscles undergo fixation over the flame of a burner or in methyl alcohol. The staining takes place for 10 minutes in isemine blue (1 gram of dye, 3 grams of phenol, 10 cubic centimeters of ethyl alcohol and 10 cubic centimeters of distilled water); then follows washing in running water and desiccation.

RESTRICTED

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In staining by the Ferrel method, the preparation is pickled twice until vapors appear in a liquid which consists of a 20 percent tannin solution in 10 parts of a saturated solution of ferrous sulfide and 5 parts of a saturated solution of basic fuchsin (the solution must ripen for several weeks). The preparation is then thoroughly washed and stained with "Fell" fuchsin while being preheated until the appearance of vapors, then washed again and dehydrated.

In staining by the Papan method, thin dehydrated smears are washed for 5-10 minutes in distilled water in a vertical position; dehydrated either at room temperature or at 37 degrees Centigrade; undergoes fixation for 24 hours in ethyl alcohol or for 5-15 minutes in methyl alcohol, and dehydrated. Then the microsections are pickled for 5-7 minutes in a well filtered Leffler solution while being heated until the appearance of vapors; then washed again in distilled water, and differentiated in absolute alcohol or in a 5 percent tannin solution for 3-5 minutes. After washing and deiccation the elementary corpuscles assume a reddish-rose hue.

10. Differentiation of Viruses

Virus infections are differentiated one from the other on the basis of clinical, pathomorphological, epidemiological, and etiological data. Not one of these indexes, taken in itself, can furnish a correct characteristic of the infection. In some cases, which are a rare exception, the etiological index plays a decisive part. In dealing with the grippe, it is impossible to delimit the illness induced by the virus of grippe Type A, from the illness induced by the virus of grippe Type B, by clinico-epidemiological and pathomorphological data. The differentiation of grippe illnesses is possible only on the basis of determining the characteristics of the precipitated virus, namely, the antigenic peculiarities of individual virus stems, their pathogenic properties with relation to animals, and their ability to induce a specific agglutination of erythrocytes.

In this chapter, without pausing for the characterization of the clinical, pathomorphological, and epidemiological indexes in the differentiation of individual virus infections, which are the subject of studies by clinical, pathomorphological, and epidemiological specialists, we will consider the principles underlying the differentiation of the inciters of these infections.

The basic indexes characterizing the inciters of virus infections, are established by way of wide and diversified investigations, such as the determination of filterability, the size of the virus, its pathogenic properties with relation to various animals,¹⁷⁵

RESTRICTED

stability to external influences, its cultivability on a chicken embryo, the determination of its morphological indexes, serological and immunological peculiarities, and the study of the pathogenesis of the infection in susceptible animals.

It is first necessary to establish the group affiliation of the individual virus, for there is no practical necessity to differentiate the virus, which strikes, under natural conditions, at the nervous system, from the viruses striking at the respiratory system of the organism. These are already sharply differentiated from one another by their pathogenic characteristics. On the other hand, the intra-group indexes of the viruses must be thoroughly differentiated, which at times is very difficult to accomplish, for instance, in the group of transmissible encephalitis.

The viruses of the spring-summer and Japanese encephalitis do not differ in size, their pathogenic properties with relation to animals show no distinct diversities, as well as their stability to the effect of physical and chemical agents. Both these viruses are cultivable on chicken embryos. They do differ by their antigenic characteristics, but even here there are group connections. These connections are expressed in the fact that, for instance, mice, immunized against spring-summer encephalitis, develop an immunity not only to this virus, but also a partial immunity to the virus of Japanese encephalitis. However, mice immunized by the virus of Japanese encephalitis, develop an immunity to the latter only, without developing any immunity to spring-summer encephalitis. There are also present partial group connections when there is a mutual

-RESTRICTED-

Table No 8.

Material for the detection of the virus

Virus infections

Rabies
 Poliomyelitis
 Spring-summer encephalitis
 Japanese encephalitis
 Encephalitis St. Louis
 Horse encephalomyelitis
 Scottish encephalitis in sheep
 Lymphocytic chorio-meningitis

Pharyngeal gargle	Skin lesions	Lymphatic and le- sions	Nervous system fragments	Spinal cord fluid	Blood
(1)	(2)	(3)	(4)	(5)	(6)
			+		
+			+		
			+	+	+
			+	+	+
			+	+	+
			+	+	+
			+	+	+
			+	+	+
			+	+	+

Speed 16 Reactions						Preferable species
Presence of histo- logical changes	Inclusions or elementary corpuscles	Neutraliza- tion test	Complement fixation test	Agglutination test	Cultivation on chicken embryon	of test animals for contamination with virus
(9)	(10)	(11)	(12)	(13)	(14)	(15)
+	+	+	+	+	+	Dogs, rabbits, mice
+	+	+	+	*	+	Monkeys, cotton field rats
+	+	+	+	+	+	Mice
+	+	+	+	+	+	Mice
+	+	+	+	*	+	Mice
+	+	+	+	*	+	Mice, rabbits, guinea pigs
+	+	+	+	+	+	Mice
+	+	+	+	*	+	Mice, guinea pigs

Preferable differentiation method

(16)

Detection of Negri corpuscles

Contamination of monkeys

Neutralization test; complement fixation test

Neutralization test; complement fixation test

Neutralization test; complement fixation test

Same as above, also pathogenic characteristics with relation to animals and birds.

Neutralization and complement fixation tests, contamination of sheep and cotton field rats.

Neutralization and complement fixation tests, pathogenic properties with relation to animals; pathomorphological investigations.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Herpes		+		+					+	+
Retrovirus in mice		+							+	+
Spontaneous encephalitis in mice				+			+		+	

serological re

Designations: + means the presence of virus, the detection of morphological changes, positive of bacteria, which are loaded with virus.

(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
+	+	+	+		+	Rabbits, Mice	Detection
+	+	+		● ○	+	Mice	Detection
+		+		+	+	Mice	Contamina

serological reactions. In column "Agglutination reaction" ● designates the agglutination of elementary

corpuscle

(16)

Detection of inclusions; contamination of rabbits into the cornea and brain.

Detection of elementary corpuscles; contamination of mice.

Contamination of animals; neutralization and complement fixation tests.

corpuscles; ○ the agglutination of erythrocytes under the effect of the virus; * agglutination

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Acute human encephalomyelitis				+	+	+		
Grippe	+							
Psittacosis	+					+		
Yellow fever						+		
Dengue fever						+		
Chappasii						+		
Mumps								+
Venereal lymphogranuloma			+					
Measles	+					+		
Smallpox		+						

(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
	+		+	+		+	Mice, rats, rabbits	Neut
	+		+	+	○	+	Mice, Skunks	viru
	+	+	+	+	●	+	Mice	Neut
	+	+	+	+		+	Mice, Monkeys	Neut
						+	Monkeys	Comp
						+	Monkeys	Comp
+				+	○	+	Monkeys	Com
	+	+	+	+		+	Monkeys, mice, cats	Neu
	+		+	+	*	+	Monkeys	Com
	+	+	+	+	○	+	Rabbits, mice	Mor

(16)

Neutralization and complement fixation tests; patho-histological investigations; stability of virus to high temperatures.

Neutralization and complement fixation test; xerithrocyte agglutination test.

Neutralization and complement fixation tests; patho-morphological investigations.

Neutralization and complement fixation tests; intra-nucleoli inclusions in liver cells.

Complement fixation test

Complement fixation test

Contamination of monkeys

Neutralization and complement fixation tests; detection of elementary corpuscles.

Complement fixation test; agglutination test, as per Sergiyev.

Morphological investigations; detection of inclusions and elementary corpuscles.

(16)

Neutralization and complement fixation tests; patho-histological investigations; stability of virus to high temperatures.

Neutralization and complement fixation test; xerithrocyte agglutination test.

Neutralization and complement fixation tests; patho-morphological investigations.

Neutralization and complement fixation tests; intra-nucleoli inclusions in liver cells.

Complement fixation test

Complement fixation test

Contamination of monkeys

Neutralization and complement fixation tests; detection of elementary corpuscles.

Complement fixation test; agglutination test, as per Sergiyev.

Morphological investigations; detection of inclusions and elementary corpuscles.

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neutralization of both viruses by the sera of animals immunized against spring-summer encephalitis.

Similar group connections also exist between the viruses of spring-summer encephalitis and Scottish encephalitis of sheep, between the viruses of acute human encephalitis, encephalitis of foxes, encephalitis of horses, and the virus of rabies, in the group of pneumo-tropic viruses, between the virus of human grippe and the virus of the grippe of swine. The virus of psittacosis has group relations connections with the virus of venereal lymphogranuloma, the virus of vaccinia--with the virus of ectromelia in mice.

The above cited group connections between various viruses must always be kept in mind when tests for the differentiation of individual viruses are conducted.

Short data characterizing the distinguishing features of individual viruses are summarized in the second part of the book. Directly below is a collated table (Table 6), indicating the preferable method for the precipitation of each virus and the methods for the differentiation of individual viruses.

See next page for Table

RESTRICTED

11. Preparation of Vaccines and Sera

Antivirus vaccines are usually a suspension of tissues from an infected animal which contain a maximum quantity of virus, or a suspension of virus infected chicken embryo tissues. In inactivated vaccines the virus is rendered harmless by the use of phenol, formalin, ultra-violet rays, or some other means. The most widely used are the formalin vaccines, and we shall dwell on the method of their preparation.

Vaccines are prepared in special enclosures under strictly maintained aseptic conditions. When working with particularly infectious material such as spring-summer, Japanese encephalitis, and others, the rules or prescriptions for laboratory procedures, where particularly dangerous infections are involved, are to be rigidly adhered to.

The division for the preparation of vaccines must consist of no less than 3 separate rooms, or cubicles, and a special vivarium. In one room, the contamination and infection (not simultaneously) of the animals takes place, in the second room--the suspending and inactivating of the virus, in the third room--the pouring and control of the laboratory made products. It is desirable to make vaccine from a large group of animals, which are worked upon simultaneously, in order to obtain a homogeneous preparation, since otherwise the virus content in the contaminated tissue may vary, and, therefore, the vaccines may turn out to be of various degrees of effect. The higher the titer of the virus in the suspension prior to inactivation, the higher the immunity.

RESTRICTED

properties of the vaccines. Therefore, only an effectively "passaged" virus with an established high titration standard is used for the preparation of vaccine.

A party of animals, by the use of the respective methods, is contaminated with the virus, and placed in a vivarium. On the day the infection takes effect, all animals are examined with the exception of those that perished more than 2-3 hours prior to examination, and with the exception of animals showing skin lesions. The animals are then anesthetized to death in the vivarium, the carcasses are immersed in a 5 percent phenol solution, each one put into a bag, and sent into the operating room for dissection. The dissection is conducted in a conventional manner. The extracted organs are placed into wide-necked tubes, or into special glass containers (if the suspending is done in blenders), or into jars containing grinding beads. The tubes and jars are covered, at the time of the dissection, with napkins wetted in a 5 percent phenol solution, and with stoppers after the dissection is done. If there is emulsification in the jars or flasks, they are covered with ground glass stoppers, wrapped into 3-5 percent lycol wetted napkins, placed into an oilcloth bag, and forcefully shaken for 15-20 minutes, until a homogeneous mass is formed.

If a blender (a homogenizer) is available, the material is transferred into it and ground. The conventional blender has an axle with legs attached to it. Special aluminum glasses containing the test material are placed under the blender axle, which, together with the legs attached to it, is lowered into the glass. During the

RESTRICTED

RESTRICTED

operation, the glass is covered with a lid and inserted into a special ring, which is tightly clamped around the glass with the aid of a screw. The axle of the agitator is connected to and driven by an electric motor at 10000-20000 RPM, causing the test material in the glass to be ground into a fine paste mass.

There are several types of such blenders, designed by the Soviet engineer Nosordin (Fig. 26).

Figure 26. Apparatus for homogenizing tissue (designed by engineer Nosordin)

The axle of this apparatus is sterilized prior to use with alcohol and formalin, then by ultra-violet irradiation. The glasses are sterilized by dry heat.

Upon grinding, part of the material is segregated for titration. In addition, the ground mass is controlled for the absence of bacterial contamination by planting into bouillon and agar-agar. Then, the initial material is diluted by a formalinized physiological solution (a 0.2 percent solution prepared ex tempore) to the required concentration, and drawn off into large flasks. The addition of physiological solution and the pumping over of vaccine must be done by using siphons only.

The vaccine is stored for a period of 10 days at room temperature, shaken up daily so that all layers of the liquid receive a uniform formalin content. Upon the expiration of the indicated time interval, the vaccine is checked for bacteriological

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sterility and absence of the live virus. For the bacteriological control, a culture is planted on bouillon, agar-agar, and semi-liquid agar-agar. The test for the absence of the active virus is effected by contaminating susceptible animals, with subsequent sacrifice. Only after all control checks were completed, the vaccine is distributed into ampules, and is ready for use.

An important defect of such vaccines is a considerable content of inert albumen. Recently, with the aid of super-centrifuging and other methods, it became possible to concentrate some viruses and purify them from extraneous albumen, and so on. In the case of epizootic, for example, vaccines from the lung tissue of contaminated mice and from the allantoic fluid of infected chicken embryo were made. And now, a method was developed for the preparation of anti-rippa vaccine from the allantoic fluid of contaminated chicken embryo, in which the virus is concentrated tenfold, by adsorption on erythrocytes.

* * *

The description of the preparation of vaccines against rabies and smallpox has long since found its way into general microbiological textbooks and manuals--we, therefore, do not dwell on them here.

In practical use at the present time are vaccines against the following virus diseases:

- (1) Live vaccines--against yellow fever, smallpox, rabies.

- 5/ -

RESTRICTED

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(2) Inactivated vaccines--against spring-summer and Japanese encephalitis, horse encephalomyelitis, epidemic grippé, Scottish encephalomyelitis of sheep, canine plague, pepparsell fever, hoof and mouth disease, rabies.

Immune Sera. Immune sera are prepared for therapeutic-prophylactic purposes and for serological analyses. An animal is immunized with an inactivated virus, if it is susceptible to the given infection, and with a virulent virus, if it is non-susceptible to the given infection. In the first case, after the repeated vaccination, in order to induce a more active development of antibodies, a virulent virus is introduced.

If a serum is designated for therapeutic purposes, it is best to immunize horses, since the serum albumen of a horse produces fewer side reactions, when inoculated into humans, than the serum albumen of other animals, and, in addition, a lot of blood can be taken from a horse. For conventional laboratory work, mostly rabbits are immunized. If sera are designated for complement fixation tests, special conditions, already described in its respective place in the preceding text, are to be maintained.

Immunization procedures may vary. Usually, 5-6 injections of vaccine, in the amount of 3-5 cubic centimeters, are administered subcutaneously or intramuscularly, at 4-7 day intervals. Then, after a 10-12 day interruption, comes the immunization with the active virus, dosages and time intervals same as above. It is recommended to begin the inoculation of virulent substance by using low concentrations. If the animal is not susceptible, the degree

RESTRICTED

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of concentration of active virus in the antigen is of no consequence. In immunizing large animals for the purpose of obtaining large amounts of sera, the introduction of antigen is made in cycles, and this procedure, together with the taking of blood from donors and the treatment of the sera, in no way differs from the preparation of anti-toxic sera.

For the conservation of immune sera chloroform may be used. In conformity with our experiment of preserving anti-grippe horse serum, the initial titration standard of the antibodies is preserved at a temperature of 10 degrees Centigrade for a year. As a rule, the period of effectiveness of a serum is determined by periodical tests for the concentration of specific anti-virus antibodies.

In the electrodialysis of horse anti-virus immune sera (grippe, encephalitis), the antibodies, as a rule, are discovered in the globulin fraction.

12. Laboratory Animals

In all virological investigations, the basic significance lies in the work performed on laboratory animals. We, therefore, consider it necessary to dwell on the basic data relating to laboratory animals and the technique connected with it. The most widely used species are--white mice, rats, guinea pigs, skunks, rabbits, monkeys, dogs, cats. Birds are sometimes used; most frequently chickens and pigeons. For special purposes, such as the preparation of immune sera, large animals, such as horses or sheep, are used.

In selecting animals for experimentation, it is always

RESTRICTED

7

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necessary to take into account the age and the weight of the animal. A healthy and normal animal usually has a definite weight corresponding to its age. In most cases, where it is necessary to effect a neurotropic infection in mice, young mice weighing 8-10 grams are the most suitable. Mice weighing 1-20 grams are less suitable for experimentation on account of natural age stability. The correlation between weight and age of some laboratory animals is depicted in Table 9.

For each species of animal there is a characteristic normal body temperature that oscillates within the limits of a definite norm, with relation to age. A rise in temperature above this norm indicates illness.

RESTRICTED

The body temperature of animals is measured either with a conventional mercury thermometer, or with the aid of more complex devices of the thermocouple type. The normal temperatures of a series of animals (taken rectally) are cited below.

Temperature oscillations as read with the aid of a conventional thermometer, may frequently depend on the depth of insertion into the rectum, which circumstance calls for an insertion to a certain depth. The normal depth of insertion in the case of guinea pigs is 3.5 centimeters. It is convenient to use thermometers equipped with a special stopping device, such as a rubber ring or a plate bulging at a pre-determined distance from the tip. Such thermometers can be inserted only to a pre-determined depth.

- 155 -

RESTRICTED

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Only animals with normal temperature are selected for tests. The existence of various spontaneous infections, with which individual laboratory animals are frequently stricken, and which may even assume an epizootic character, must always be born in mind. In the case of mice and rats, in addition to bacterial infections, such as septicemia, paratyphoid, trichinosis, there may be encountered spontaneous virus infections (necrosis, spontaneous encephalitis, virus pneumonia).

In the case of guinea pigs, the most propagated infectious diseases are pneumonia and bronchial pneumonia, pseudo-tuberculosis, paratyphoid, strepto-bacillary dentitis. Rabbits may become afflicted with pasteurellosis, pneumococcosis, bacillariosis, pseudo-tuberculosis, coccidiosis, leishmaniasis, spontaneous encephalitis. Other animals may be afflicted with other diseases. This entire problem is discussed adequately in special articles and textbooks. It is necessary to emphasize that only normal and healthy animals, bred in special nurseries, are selected for the laboratory virus vivaria, it being the case that the possibility of spontaneous infection is always taken into account.

In addition to operating instructions with relation to experimental animals, which were furnished in the Chapter devoted to virusological research methods, general data will be cited here on various methods and procedures for the contamination of animals for experimental purposes.

Prior to contamination, every animal is weighed. If the experiment requires a large number of mice, they are all taken of approximately the same age, consequently, the same weight. Each animal

RESTRICTED

RESTRICTED

is to be tagged.

Small metal number plates are used for rabbits and guinea pigs, and they are inserted into the outer ear of the animal. Mice and rats are marked by dyeing small areas of their wool in different colors. The most stable and practical dyes for this purpose are a 0.5 percent solution of fasten "Grell" and a water solution of picric acid. It is convenient to adopt a definite dyeing diagram which makes it possible to use only one dye in the marking of 9 animals, and only two dyes in the marking of 100 animals.

The following dyeing diagram is used in our laboratory: the conventional numbers identifying units (Figure 27) are dyed with fasten, while the conventional numbers identifying tens are dyed with picric acid. An animal dyed with fasten will bear the following conventional numbers in the form of spots in conformity with respective parts of the body: left anterior paw--No. 1, left side--No. 2, left posterior paw--No. 3, hind--No. 4, back--No. 5, wool at the tail beginning--No. 6, right anterior paw--No. 7, right side--No. 8, right posterior paw--No. 9. If dyed with picric acid, the same parts of the body will signify, respectively, the following numbers: 10, 20, 30, 40, 50, 60, 70, 80, 90. It can be seen that by dyeing each of the above enumerated parts of the body with two colors, 99 animals can be marked. If the anterior left paw has two color spots alongside each other, yellow and red, the animal will be No. 11; if the same left paw has a yellow spot only, and the adjacent left side--a red spot, the animal will be No. 12, and so on.

RESTRICTED

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For complex methods of contamination requiring surgery, the animals must be fixed in place. Various types of boards, stands, and supports are used for this purpose. In all cases when the operation is painful, it is necessary to resort to narcosis. In order to narcotize guinea pigs, mice, and rats, they are placed in a glass jar that can be closed, or under a glass hood, with a small piece of ether saturated cotton underneath. Rabbits and larger animals are administered the ethereal narcotic through a mask. Conine is also used at the present time, in intravenous narcosis in which various barbituric compounds are used. Narcotic gases are also used successfully in our laboratory.

Subcutaneous contamination. In subcutaneous contamination of mice and rats the most convenient method of injection is the introduction of the needle under the skin of the spine at the occiput. This is the accepted method in our laboratory, and the operation can be performed by one man without an assistant. The mouse or rat is fixated, its tail is clamped between the fourth and fifth fingers of the left hand; with the thumb and index finger the skin fold on the neck is stretched, while the middle finger slightly presses the animal toward the supporting table. The skin is disinfected with iodine, and the injection administered by the right hand with the tip of a syringe needle. The dosage for mice are up to 0.5 of a cubic centimeter, for rats--up to 3 cubic centimeters (figure 28 which is a photo).

In intramuscular contamination, a spot is selected where the muscular layer is developed best, most frequently the muscles of the

RESTRICTED

RESTRICTED

thigh. The area of the skin around the injection spot is made ready in the same manner as for a subcutaneous injection.

Intradermal contamination. The skin is thoroughly cleared of hair and washed with alcohol. The contamination is effected with a tuberculin syringe equipped with a thin sharp needle. The needle is forced into the thick of the skin, yet almost parallel with the surface so that its point is discernible through the epidermis. The injected material lifts the surface layer of the epidermis in the shape of a bubble. The dose is 0.1-0.2 of a cubic centimeter.

An intradermal infection can also be effected by rubbing the contaminative substance into a scarified area of the skin. To accomplish this, the selected skin area is thoroughly cleared of hair, washed, and scarified. Then, a drop of the contaminative material is rubbed into it with a glass rod or spatula.

Intraperitoneal contamination. Areas of skin on the abdomen, somewhat above the symphysis [pubis] are cleared of hair and treated with alcohol and iodine. The assistant holds the animal in a vertical position, head downward; in this position, the internal organs in the abdominal chamber are somewhat shifted, and the danger of injury to the intestines from the injection needle is lessened. A small incision is made in the skin with a small sharp scissor, through the incision a blunted syringe needle is inserted, moved in the direction between the skin and the muscles to a depth of one centimeter, and only then a perforation is made into the abdominal chamber. Mice and rats receive the injection without a preliminary incision of the skin in the iliac area.

RESTRICTED

the moment of penetration of the needle into the abdominal chamber becomes known to the operator by the vanishing of resistance under his hand. The contaminative substance is introduced in a dose of 1 cubic centimeter for mice, 2 cubic centimeters for rats, 5 cubic centimeters for guinea pigs, and up to 10 cubic centimeters for rabbits. Upon the withdrawing of the syringe needle, the skin becomes shifted to its original place, and the perforation into the abdominal chamber closes itself.

For an intravenous contamination of mice and rats, one of the veins running along the tail is used. This vein becomes visible when the tail is preliminarily kept in warm water (at a temperature of 40-50 degrees Centigrade). The assistant is to hold the animal in a fixed position and exert a slight pressure upon the tail at its root, in order to retard the flow off of the blood. The operator holds the tail in a fixed position with his left hand, forces a thin syringe needle at an acute angle into the vein at the lower third of the tail, where the skin is thinner, and presses slightly upon the piston of the syringe (Figure 29, which is a photo). If the piston moves easily, and the vein becomes pale along its entire length, the injection was effective. The pressure by the assistant upon the vein at the root of the tail is, of course, discontinued during the injection. The maximum dose is one cubic centimeter for mice and two cubic centimeters for rats.

..... In the case of guinea pigs, the injections are administered into the v. saphena on the inner surface of the muscle over the shin bone. This, however, can only be done in the case of white guinea

RESTRICTED

plex, since the vein is not visible through dark skin. An injection can also be made into the jugular vein, but this requires a preliminary skin incision.

On account of these difficulties, guinea pigs most frequently receive the injection directly into the heart. The heart impulse is felt for at a point one centimeter above the handle of the breastbone, and somewhat to the left of it, between the ribs, a needle without a syringe is guided in. As soon as the needle penetrates into the heart, blood will become visible in its orifice. The syringe is then connected to the needle, and the contaminative material introduced slowly (not more than 2 cubic centimeters).

The least difficulties are encountered in the contamination of rabbits. The injections are made into the auricular vein of the ear. The hair along the edge of the ear is plucked out, the skin is washed and disinfected. In order to induce the swelling of the vein, the ear is slightly tapped on, and the vein at the root of the ear compressed. The ear is placed on the index finger of the left hand, and with the right hand a thin needle syringe is introduced almost parallel to the plane of the ear. As soon as the needle is in the vein, it is moved somewhat ahead along the vein and pressed together with the vein by the fingers of the operator. If the needle has penetrated into the vein, the liquid from the syringe will enter the vein without any effort, and the vein becomes pale along its entire length. When the injection is completed, the vein is compressed below the perforated spot, and the needle is extracted. The perforation spot is compressed with dry sterile cotton. In order to be

RESTRICTED

RESTRICTED

able to use the same vein for several injections, it is necessary to extend the injections as far as possible from the root of the ear, and the perforation spot is not to be rubbed with alcohol, since this induces the formation of blood clots and the tanning of the skin. The dosage for the intravenous contamination of rabbits is up to 1 cubic centimeter (Figure 30, which is a photo).

In intravenous contamination, it is necessary to see to it that the contaminative material contains no large particles, liable to cause embolism, and that the syringe does not contain a single air bubble.

Contamination through the nose is conducted under etheral narcosis. It is very important that the respective rules for the administration of the narcotic are strictly observed. The ether is to be administered in large quantities for short periods, as a result of which the sleep induced is not long but deep.

Large animals receive the narcotic individually, most frequently with the aid of a mask. Mice and rats are placed in a tightly closed glass jar, at the bottom of which there is a piece of ether saturated cotton. Through the glass of the jar the animals can be observed. As soon as the animal is asleep, the mask is removed, or the animal is taken out of the jar, and the contaminative material is introduced with the aid of a thin Pasteur pipette or syringe, slowly, drop by drop (See Figure 31, which is a photo). When the narcotic is fed correctly, the animal sleeps well and inhales deeply the infectious material which penetrates directly into the lungs. When the narcotic is weak, the animal, by coughing, tries

RESTRICTED

RESTRICTED

to force the substance out of the respiratory passages. This circumstance is to be taken into account, and laboratory personnel is to wear masks and be behind a protective glass during the time the infectious material is being administered. When the narcosis is too deep, the breathing of the animal becomes shallow, and the contaminative material is not inhaled. In addition, over-narcotized animals develop emphysema of the lungs, and most of them perish within the first 24 hours upon extubation. Animals must also not be extubated with over-concentrated suspensions, since most of them perish within the first 24 hours from the toxic effect of tissue suspensions. However, extubation with over-diluted suspensions is not always successful. It is, therefore, recommended to use suspensions in concentrations not higher than 1:10 and not lower than 1:1000. The dosage for mice is 0.03-0.05 cubic centimeter, for rats 0.05-0.1 cubic centimeter, for guinea pigs and rabbits up to 2 cubic centimeters.

Contamination into the anterior eye chamber is successful in the case of large animals only, such as dogs, rabbits, and more difficult in the case of guinea pigs.

The animal is affixed upon the operating table, with its back upward. Several drops of 3 percent cocaine are placed upon one eye, and as soon as the anesthetic takes effect, the assistant, with the aid of a blunt instrument, slightly rolls the eye out from the socket, and the operator, using the left hand, grasps the conjunctiva with thin eye pliers, in order to immobilize the eye ball, while with his right hand, he perforates the cornea at the limbus with

RESTRICTED

RESTRICTED

a thin needle, which is moved in a central direction until the liquid flows in its orifice. After several drops of the liquid from the exterior chamber of the eye will flow off, the syringe is mounted onto the needle, and the contaminative material pumped in, in a dose not to exceed 0.05 cubic centimeter.

Contamination onto the sclerified cornea is effected by first anesthetizing the eye with a few drops of cocaine, then effecting several scratches of the cornea with a sharp needle, and placing the contaminative material upon the sclerified cornea.

Intracerebral contamination on large animals is conducted through the trepanation opening. For each operation, the animal is tied to the operating table or to a special stand, with its back upward. No ether should be used as a narcotic, since the mask will be in the way. The trepanation area is cleared of hair and treated with iodine. The skin is stretched taut, and, with the aid of a scalpel, a 1-2 centimeter slash is made parallel to the longitudinal skull axis, somewhat above the line connecting the rear angles of the eyes and more lateral than the middle line. The periosteum is then dissected, and the trepanation accomplished. A syringe needle is inserted through the opening formed in the bone, the hard cerebrospinal membrane is perforated, and with a slight pressure upon the syringe piston, 1-2 drops of the contaminative material is inoculated into the brain. The opening in the bone is then covered by the skin that shifted back into place, and sutures made in the skin wound. This method of contamination is used on dogs and rabbits.

RESTRICTED

A method of intra-cerebral contamination of rabbits without preliminary trepanation is used successfully in our laboratory. It is effected through the sulcus supraorbitalis, where the bone is thin and allows the needle to pass rather freely. The assistant affixes the rabbit's body, while the operator, with his left hand, presses its head onto the table (made comfortable by a soft covering), and with his right hand he effects the perforation through the above described and preliminarily located spot, which has been cleared of wool and treated with iodine. The perforation is made with a special short needle (4-5 millimeters long), after the skin was gathered up from the middle toward the sulcus supraorbitalis. This is done in order to facilitate the closing up of the perforation made in the cranium. The direction of the needle in the act of perforation is not vertical, to avoid penetration into the eye socket, but somewhat inclined toward the middle line. This method of contamination is very convenient, since it traumatizes the animal to a smaller degree, shortens the time of the operation, and is less liable to cause suppuration. Rabbits can take their operation without being narcotized. Guinea pigs are contaminated intra-cerebrally in the same manner, with or without preliminary trepanation (See Figure 32, which is a photo).

The intra-cerebral contamination of smaller animals, such as rats and mice, is effected with a tuberculin syringe with a thin needle, upon which a metal sleeve is affixed in such a way that the truncation of the needle is completely exposed. This insures the same depth of intra-cerebral penetration in all inoculations. The mouse is held in a fixed position with the left hand so that the

RESTRICTED

RESTRICTED

RESTRICTED

thumb and the index finger will stretch the skin of the head toward the occiput, and the tail is fixed between the little finger and the ring finger. The skin over the parietal bones, to the sides of the sagittal suture, is treated with a one percent iodine solution, and the perforation is made here. The contaminative material is to be introduced gradually, in order not to induce a sharp increase in the intra-cranial pressure.

Contamination into the large cistern by a sub-occipital perforation can be used on large animals only (rabbits, dogs). In small animals, the space between the membrane and the brain is too small to allow such a procedure. The rabbit is fixed tightly on the operating table, belly upward, with the humeral belt at the level of the table edge, and with the neck and head loosely lowered. The assistant grabs the head of the rabbit with both hands so that his index and middle fingers are under the lower jaw, and his thumbs are thrust against the parietal bones. He then bends the rabbit's head in such a way as to form a right angle between the plane of the body and the neck (Figures 33 and 33a, which are photos). With such a position, the distance between the first vertebra of the neck and the skull is increased, and the ligament connecting the two is stretched. The wool over this area of the head, between the occiput eminence and the osseous extension of the first vertebra of the neck, is shaved off and treated with alcohol and iodine. The perforation is effected in the exact center between the above two, holding rigidly to the center line. A slight deviation to the right or to the left will unavoidably bring the needle into the surrounding veins. The perforating needle is to be adequately fine, but not

RESTRICTED

RESTRICTED

truncated at an acute angle, bearing in mind that the distance between the cerebral membrane and the cerebrum equals to 1-2 millimeters. To avoid the clogging up of the needle in passing through the soft tissues, it is best to insert a mandrin into it. The needle is to be guided with caution. In passing through the hard cerebral membrane, a slight crackling sound becomes audible, which is to serve as a stop signal. As soon as the spino-cerebral fluid becomes discernible in the orifice, a dry sterile syringe, in the chamber of which a negative pressure is created by the movement of the piston for the purpose of drawing the spino-cerebral fluid, is mounted over the needle. The extraction of the spino-cerebral fluid is to proceed slowly so as to avoid an abrupt decrease in intra-cranial pressure.

From 0.5-0.7 cubic centimeter of spino-cerebral fluid is extracted. Then, another syringe, containing no more than 0.5 cubic centimeter of the contaminative material, is mounted on the needle and the inoculation completed.

Contamination into the peripheral terminations of nerves.

For this purpose the sciatic nerve is the one that is used most frequently. The animal is affixed to the operating table with its back upward. The rear surface of the thighs is cleared of hair and disinfected. Either general or local anesthesia is used. A gash 3-4 centimeters long is made in the skin, the muscles of the thigh are moved apart with a blunt instrument, thereby exposing the nerve. Thin gauze surgical pads are inserted under the nerve, lifting it from the depth of the wound and preventing the contaminative

RESTRICTED

RESTRICTED

material from coming into contact with the surrounding tissues. A perforation is made into the nerve with a fine tuberculin syringe needle held at a very acute angle, and a small dose of the contaminative material is introduced. The needle is removed carefully so as to prevent the inoculated material from backing up. The skin is sewn up with silk and clamped.

General Rules of Procedure for Virological Laboratories.

Virological laboratories are organized along the lines of conventional bacteriological laboratories, but with due consideration given to the peculiar and specific requirements involved in virological research. The principal condition to be scrupulously observed is the cleanliness of the premises, which is obtained, in addition to ordinary maintenance and hygiene, by periodical treatment of the premises with disinfectants, such as phenol, lyso, calcium hypochlorite, or with steam and ultra-violet irradiation (See Figure 11, which is a photograph). The laboratories must be well exposed to daylight and free of unnecessary furniture.

In addition to equipment and ware available in any bacteriological laboratory, virological laboratories must be equipped with adequate quantities of porcelain beaters, special jars with grinding beads, and blenders, or homogenizers for the pulverization of contaminated tissues. All these implements must be well sterilized prior to use; the beaters are to be equipped with h-ply gauze coverings.

Do precipitate coarse particles from pulverized virus

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containing tissues, centrifuges capable of 2,000-3,000 RPM must be available. For the complete purification of the virus from inert substances and for its concentration, centrifuges capable of not less than 10,000 RPM must be available. Taking into account the fact that viruses survive better at low temperatures and that temperature oscillations may prove lethal, the availability of a refrigerator with a constantly maintained temperature of not higher than 5 degrees Centigrade is a prerequisite for any virological laboratory. It is best to use electric refrigerators, where a constant temperature is maintained automatically, or a cooler regularly charged with ice. In the absence of such as, for instance, under field conditions, hermetically closed boxes packed with ice may be used. It should be noted that even during the tests on the laboratory table the virus containing material is to be kept in containers with ice. It is best to maintain the viruses at minus 60-until 70 degrees Centigrade, which temperature is attained by the use of dry ice (solid carbon dioxide). Where there is the possibility of regular shipments, about 10 kilograms of solid carbon dioxide should be sent to the laboratory daily. The solid carbon dioxide can be kept in boxes lined with a thick layer of heat insulating material.

Each laboratory is to be equipped with an apparatus for the desiccation of the viruses in vacuum. An individual thermostat for each laboratory is desirable, yet several laboratories may simultaneously use a single thermostat in common. All virus containing material is to be controlled for the absence of bacteriological pollution by planting onto various nutritive media and

RESTRICTED

RESTRICTED

incubation in a thermostat for no less than 24-48 hours.

Since the source of viruses in the laboratory are the various tissues and organs of stricken animals, it is necessary to have a set of various special instruments: forceps of various sizes, tooth clamp, surgical needles and needle holder, syringes, spatulas, etc. These instruments are used for the contamination and dissection of animals, extraction of infected tissues, and the like.

It is obligatory to sterilize all instruments prior to each procedure in special sterilizers.

There must always be on hand an ample supply of the following: physiological solution and bouillon for the preparation of suspensions, 10 percent glycine solution for the preservation of the viruses, nutritive media for bacteriological control (agar-agar, bouillon), alcohol for fumigation through which instruments are passed, ether or chloroform for anesthetics, and antiseptic compounds (phenol, lyso) for disinfecting instruments, premises, and furniture before and after work.

Work with viruses, particularly dangerous as to the possibility of laboratory contamination, also with virus carriers, is permissible only in specially adapted premises that satisfy the requirements of complete isolation and safety, and also provided with all possible means of labor protection for the laboratory personnel. For work with viruses of transmissible forms of encephalitis, an obligatory procedure, to conform with the special instructions of the Ministry of Health, USSR, was established, and the work with such

RESTRICTED

RESTRICTED

viruses and their carriers is to be done only in institutes and laboratories, which have a special licence issued by the Anti-Epidemic Administration, Ministry of Health USSR.

Laboratory room, where viruses pathogenic to humans are under observation, must be well lighted and consist of two compartments separated by a glass partition (see Figure 36, which is a photo). The inside compartment is a box of the operating room type, and contains a laboratory operating table and the necessary work paraphernalia: a sterilizer for instruments, a vat for the contaminated material, a jar with lysol for hand washing, a vat filled with lysol for putting up the dead animal, etc. The outside compartment serves for the changing of special clothing and auxiliary procedures. The walls of the laboratory room are covered with oil paint, and it is advisable to have a tile panel up to a height of 1.1 meters. The floor is to be covered with linoleum. At the entrance to the operating room, a spill rug saturated with a 5 percent phenol or lysol solution is placed.

The actual work with the contaminated material is done on the operating table covered completely with a smooth linoleum and padded with tin sheet or glass plate (Figure 37, which is a photo). The operating spot is covered with several layers of gauze saturated in a 5 percent lysol solution. The lysol protected surfaces are only for the placing of objects which are directly connected with the test, such as the animals undergoing dissection, test tubes with tissue suspension, etc. All the instruments and other requisite objects are to be to the side of the operator.

- 171 -

RESTRICTED

RESTRICTED

When work is completed, the gauze is immersed into a jar containing lysol, and the table is wiped with a 5 percent lysol solution.

All the work, with the exception of the contamination of large animals, is to be performed from behind a protective glass, which protects the operator from drop contamination (Figure 38, which is a photo). The glass partition is disinfected by wiping it with a disinfecting solution.

All personnel is to wear smocks and helmets. Each laboratory worker is to have two smocks buttoning from behind. One smock is for ordinary jobs around the laboratory, the other one to be used in the operating room only. Smocks are to be changed as needed, but not less than once every 3 days. The soiled smocks are placed into metal containers, and, as they accumulate, they are sent to the disinfection chamber.

In the operating room two smocks are to be worn with a rubber apron and cuff shields, rubber gloves and gauze masks (4-ply gauze over mouth and nose), and protective goggles. When work is done from behind a protective glass, goggles may be dispensed with.

Rubber gloves are disinfected by a two-time immersion of gloved hands into a jar containing a 5 percent lysol solution.

Gloves may be taken off only after hands were immersed in lysol. After taking them off, they are decontaminated by immersing for 30 minutes in a 3 percent lysol solution, and the hands are washed with soap. The decontaminated gloves are washed in running water, dried, checked for holes, powdered and placed into

RESTRICTED

RESTRICTED

a clean jar. The use of torn gloves is forbidden.

Sterile gauze masks are changed two or three times during the day. A gauze mask that was taken off is not to be used again, but sent for sterilization.

After goggles are removed, they are to be wiped with lysol and kept in a closed jar on the table. The taking off of masks and goggles is allowed only after the hands are decontaminated in a disinfecting solution.

When work is completed, all instruments are sterilized in boiling water, and all objects are carried from the table, after being sterilized outside, into a closet or into a sterilization vat.

Containers with contaminated material, after use, are placed on the spot into a vat, which, after the addition of soap, is covered with a lid, locked, and sent to the autoclave.

Prior to autoclaving, the vat is half filled with water through a funnel inserted into a hole in the lid. The funnel is disinfected by immersion into lysol. If, due to the nature of the containers, undergoing decontamination, autoclaving is not appropriate, they are to be disinfected by boiling.

The carcasses of dead animals are placed into small vats. If there are no facilities for the cremation of the carcasses, the small vats containing them are autoclaved.

All objects carried out from the operating room, including

RESTRICTED

the cages containing mice, are preliminarily wiped from the outside with lysol saturated gauze.

A laboratory worker, who met with a mishap (the breaking of a container with virus suspension, or the spattering of the latter), is to remain in his place, call for the laboratory attendant and the charwoman with all the paraphernalia for the disinfection and removal of the contaminated material. The decontamination of the mishap area is effected by the laboratory attendant wearing rubber gloves, rubber shoes, rubber shoes and a double smock. The surface, upon which the virus suspension was spattered, is flooded with a 5 percent lysol solution.

The liquid part of the contaminated material is gathered up with hygroscopic cotton held by a pincers, and, together with the latter, placed in an enamel tray. Fragments of containers are picked up with the aid of pincers and a trowel into the same tray, which is placed into the appropriate vat.

If the contaminated material found its way into the eye or onto a mucous membrane, it is imperative to wash the effected spots with a one percent lysol solution or warm soap water. In addition, a prophylactic intra-muscular inoculation with specific immune serum over a period of 2-3 days, is recommended. Generally, all personnel serving in the laboratory is advised to undergo a prophylactic vaccination with the respective vaccine.

For the maintenance of test animals, the laboratory is equipped with a vivarium. The proper maintenance of test animals

RESTRICTED

is of great importance and insures the success of virological research. The cubicles of the vivarium are to be impenetrable for rodents and are not to be crowded with cages, the latter to be placed on special shelving, at a height not less than 0.5 of a meter from the floor (Figure 39, which is a photo).

It is necessary to maintain in the vivarium a constant and favorable temperature, not below 15-17 degrees Centigrade. The vivarium premises are to be isolated from other rooms, and they are to insure complete safety to the service personnel, and also preclude the possibility of contamination as between animal and animal. To comply with the latter condition, separate cubicles are to be provided for animals contaminated with viruses, which may propagate by the drop and contact methods.

In order to avoid the occurrence of spontaneous infections, such as ectromelia in mice, spontaneous encephalitides in mice and rabbits, bacterial infections (paratyphoid, tularemia, and others), the animals shipped to the vivarium are to come from special nurseries or colonies that were checked for the absence of epizooties. In addition, all the animals, prior to being accepted in the vivarium, are subjected to a two-week quarantine.

Small laboratory animals (mice) are placed into small cages or into 10-20 liter glass jars with wide openings covered with mesh lids (Figures 40 and 41, which are photos). Rabbits, guinea pigs, and rats are maintained in wooden or iron cages with tight-fitting galvanized or iron trays, which are disinfected daily with a 5 percent solution of lysol, phenol, or washed in boiling water, then

- 75 -

RESTRICTED

RESTRICTED

thoroughly wiped and dried (Figure 41, which is a photo). Dry sawdust is spread on the bottom of the jars or cages to serve as bedding. Large animals located outside of the laboratory for purposes of immunization, are also kept in isolated enclosures under conditions insuring safety during the tests with contaminated materials.

Each cage containing test animals must have a label bearing the number of the cage, the name of the test, the date, the sequence numbers, and the quantity of the animals.

The same data is carried into a special daily registration journal, where the number of animals in the cages is recorded.

The cages and jars are cleaned daily. In the case of particularly dangerous infections, the caretaking personnel are to wear double smocks, rubber aprons, cuff shields, gloves, rubber overshoes, and masks, as was indicated above.

The cages are cleaned with a sheet iron scraper, the trash is collected into a tray placed edgewise into the cage. The trash from the cages, also the dead animals are thrown into a vat. The trash filled vat is disinfected in an autoclave, and only then the trash is thrown into pits or burned. The animal carcasses are to be unconditionally cremated.

After all the cages were cleaned, the workers must disinfect their hands and tools in a 3 percent lysol solution or a solution of caustic alkali.

The cage that becomes vacant after the test is completed is disinfected or preferably autoclaved. Feed troughs and drinking

- 176 -

RESTRICTED

RESTRICTED

containers are disinfected in boiling water. Every 2-3 weeks the entire vivarium undergoes a special disinfection by ultraviolet irradiation, and by DDT spray of the floor and shelving.

The maintenance and care of the animals is to be under the supervision of a veterinary. All experimental animals are to receive an adequate amount of food and drink.

- 177 -

RESTRICTED

RESTRICTED

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PAGE TWO

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A whole series of inhibitors of virus disease, starting primarily at the central nervous system, is incorporated into the group of neurotropic viruses. The designation "neurotropic viruses" is not altogether correct for this group, since, strictly speaking, only the viruses of rabies and poliomyelitis are trouble to nerve tissue. These two viruses strike selectively at the cells of the central nervous system, is not appropriate outside of the latter, and, as a rule, are not found in the blood of the patient. The neurotropic character of other viruses of this group are determined by the fact that they strike at the central nervous system only preferentially, and, together with that, they strike at other organs and tissues, and are regularly discovered in the blood. The virus of spring-summer encephalitis is relatively neurotropic, and it strikes not only at the nerve tissue, but at tissues of mesodermal origin. Pathologic-anatomical examinations in cases of spring-summer encephalitis show continuous transmittations in the abdominal organs, the liver, the kidneys, and the heart. The virus is discovered not only in the brain of the encephalitis victim, but also in the blood and in the internal organs. It is easily propagated in tissue cultures and in a developing chicken embryo.

-17-

RESTRICTED

neurotropic viruses induce diseases of the central nervous system, of various forms or intensity, stimulating there is a wide variety of their manifestations, beginning with a relatively light infection, such as lymphocytic choriomeningitis, and ending with a grave and lethal. In the case of humans, infection, such as rabies.

It should be emphasized that the clear differentiation of the virus diseases of the central nervous system, on their clinical and pathological-anatomical aspects, is not always possible. Under the action of the same factor, clinical manifestations may vary from abortive forms, accompanied by a slight infection of the nervous system, to pathological processes of a very serious nature accompanied by irreversible lesions. This takes place in poliomyelitis, spinal-cord encephalitis and in other neuro-virus infections.

It is also known that different viruses frequently induce similar pathological processes. This, first of all, relates to the virus encephalitides, in the case of which a clinical and anatomical differentiation is not always possible, particularly under experimental conditions with animals.

Below there is a brief description of the group of neurotropic viruses, composed of the viruses of rabies, poliomyelitis, encephalitides, and lymphocytic choriomeningitis. In addition to these, virological literature mentions some others, the special description of which is left out here since they have no practical significance. One of such is the so-called virus of the western Nile, which was precipitated from the blood of

as streptococcal, showing many neurological symptoms. Its
antigenic properties, this virus is close to the viruses of
encephalitis St. Louis and Japanese encephalitis. However, no
epidemic cases of herpes, being subsequently detected by this
virus, are on record. Thus, the identification of the virus of
encephalitis west-nile must be considered as causing, at least
any other form of encephalitis.

There is on record the virus of Australian encephalitis,
or B-herpes, which was observed in 1914-1915 in some species
of Australia, was also identified by the antibody tests
of encephalitis. The same statement was observed
in 1914-1915, but it was observed later, and its
virus was identified. In 1915, the virus of B-herpes
was identified. The virus of the infection of this
herpes was identified. The only fact that was established
and its relationship to the virus of encephalitis, and
on record.

It is also necessary to mention encephalitis herpes.
Numerous attempts to precipitate the virus of the encephalitis
encephalitis herpes, which struck hard in various countries
at the end of World War I (1914-1918), ~~many attempts~~
were not successful. Nor was the theory, to the effect that
the virus of herpes had an etiological significance in the
above epidemic, ever corroborated.

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General Data on Infection

Measles is an acute infectious disease of the central nervous system that can be transmitted to humans by contaminated wild or domestic animals.

The incubation period in humans is very irregular, is a long term, but is rarely in excess of 21 days, and averages of 12-14 days.

Infectious disease is an extremely early disease, and the prodromal, as a rule, is harmless. Clinically, there are 3 stages of the disease. The stage of the prodromal symptoms of the disease, of the prodromal symptoms, is practically always characterized by the setting in of pain at the location of the site, of the prodromal symptoms, such as a rise in temperature, headache, nausea, nervousness, irritability, and a general depression. These prodromal period symptoms are gradually accelerated and transformed into the stage of the prodromal disease, accompanied by the appearance of new symptoms, evolving with the development of the pathological process in the nervous system. Spasms of the degenerative muscles set in, and the patient is afraid to drink during asthma (dyspnea), attacks of respiratory spasms, joined by general tonic convulsions, take place, and the general state of agitation becomes extremely acute. In most cases, the stricken victim expires amidst spasmodic convulsions. If the acute phase of the disease has lasted more than 3 days

CLINICAL DATA

General data on infection. Rabies is an acute infectious disease of the central nervous system that can be transmitted to humans from contaminated wild or domestic animals.

The incubation period in humans may vary from 10 to 60 days, but is rarely in excess of 90 days, and averages on average at 35-40 days.

Rabies in humans runs an extremely heavy course, and the prognosis, as a rule, is hopeless. Clinically, there are 3 stages of the disease. The stage of the prodromal or premonitory disease, or the prodromal symptoms, is practically always characterized by the setting in of pain at the location of the bite, and also by general symptoms, such as a rise in temperature, headache, nausea, nervousness, irritability, and a general depression. These prodromal period symptoms are gradually accelerated and transformed into the stage of the pronounced disease, accompanied by the appearance of new symptoms, evolving with the development of the pathological process in the nervous system. Spasms of the deglutitory muscles set in, and the patient is afraid to drink fearing asthma (dysphagia), attacks of respiratory spasms, joined by general tonic convulsions, take place, and the general state of agitation becomes extremely acute. In most cases, the stricken victim expires amidst spasmodic convulsions. If the acute phase of the disease has lasted more than 3 days

there frequently develops the third stage of the disease, which manifests itself in the paralysis of various groups of muscles, frequently running true to the type of acute ascending paralysis (Landry's paralysis).

Patho-histological investigations are made up of vascular inflammatory and degenerative changes in the gray matter of the cerebrum and the spinal cord, and are manifested by considerable perivascular infiltration of the cerebral tissue, by the proliferation of glioblasts and microglia, by sharply accentuated degeneration of ganglionic cells and neurofibrilia. The presence of special formations, the so-called neurocytes, are specific in the case of rabies, and they are principally disposed in the cytoplasm of the ~~neurons~~ ^{neurons} of the brain. Fully developed neurocytes are usually found in the ganglionic cells of the thalamus, in the pyramidal cells of the cortex of the brain, in the Purkinje's cells of the cerebellum, in the ganglionic cells of the medulla oblongata, the medulla oblongata, and the spinal cord.

Precipitation of the virus. Beginning with 1890, investigations by Pasteur established the possibility of the experimental contamination with rabies of dogs, rabbits and other warm-blooded animals. It was also Pasteur who proved that the virus of rabies is invisible through a microscope, and that it cannot be cultivated on artificial nutritive media. The virus of rabies precipitated from a stricken dog, after a series of cerebral passages on rabbits, has acquired the

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properties of inducing the disease in these animals, after a definite and constant incubation period. Such a virus was named by Pasteur a fixed virus, in contrast to the conventional virus of street rabies.

The fixed virus was used by Pasteur for the preparation of an anti-rabies vaccine. It is now 60 years since this fixed virus is being passed through the brains of guinea pigs. Periodically, many more strains of the fixed virus of rabies were registered from stricken animals at various times in various parts of the world.

The virus of street rabies is characterized by the fact that it induces a prolonged and vacillated incubation period and the formation of specific inclusions in the brain. In the intra-cerebral inoculation of rabbits, the incubation period is usually 12-14 days, but sometimes it attains the duration of 90 days.

The fixed virus is characterized by a short incubation period (usually 3-6 days following the intra-cerebral inoculation of rabbits), by the absence of typical inclusion, and the homogeneous course of the disease, frequently resulting in paralysis.

Pathogenic qualities for animals. The virus of rabies is pathogenic for all warm-blooded animals. Contamination cannot be effected through the undamaged skin or through the alimentary

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in rabies-stricken animals, the virus, in addition to the central nervous system, is present in the salivary glands of the animals, and frequently in high concentrations. In dogs, for example, the virus of street rabies, inoculated experimentally into the masticatory muscles, was discovered in the salivary glands. in 39 cases out of 67 (58 percent). In rare cases the virus was discovered in the pancreas and in the kidneys. The blood, the bone marrow, the spleen, the

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~~Microscopic~~ permeance of the intestines, were all free of the virus.

Filterability, Size, Morphology. The virus of rabies passes through a Berkefeld V filter, passes irregularly through Millipore, and is filterable. It is also difficult to obtain a virus filter to which many, or most, filters of the type used in virus filters.

The size of the virus of rabies particles is determined with the aid of ultra-permeation filters, and is found to be 100 millimicrons. Due to the fact that the sizes of the particles of the virus of rabies are relatively large, an attempt was made to detect this virus microscopically. This search was made difficult by the localization of the virus in the cells of the central nervous system, and to date no confirming data, with respect to this, has been obtained.

As was already mentioned, in rabies, Negri corpuscles are found in the cells of the central nervous system. The Negri corpuscles are specific, they are easily stained, and their detection is significant in the serodiagnosis of rabies. However, these formations are not always detected in the cerebral tissue of stricken animals. The presence or absence of inclusions primarily depends on the duration of the sickness. Most frequently the Negri corpuscles are discovered during the first days of the sickness.

... ..
In appearance, the inclusions are sharply outlined round, oval, or elongated eosinophilously stained corpuscles 1-3 microns in diameter. The inclusions are disposed in the

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cytoplasm of the cells, a typical corpuscle contains evenly
uniformly stained granules, 0.2-0.5 micron in diameter, and is
surrounded by a lipid zone, which is clearly discernible in
electronically stained preparations.

Cultivation of the virus. The virus is preserved on and
propagation of the virus of rabies was obtained successfully in
tissue cultures, made up as a tissue solution, with the addition
of 5-10 percent of human or monkey serum and a mixture of amino
acids. Other tissue cultures containing the
embryonic tissue of chicks, rats, rabbits, were also tried
as controls. It must be noted, however, that in high con-
centrations of virus the cells become very sensitive.

The cultivation of the fixed virus of rabies on the
chick embryo cells of chicken embryos was successful with
1-4 day old embryos. It is desirable to collect a series of
passages of the fixed and street viruses of rabies by
continuing the chicken embryos here-continuing. Most of
the embryos retain their viability in this process, are active
in the presence of the virus in their brain, and, in the
case of infection with the virus of street rabies, the
presence of a considerable amount of neutral corpuscles. There
is a description of recently conducted successful tests for
the cultivation of the virus on a chicken embryo after
preliminary passages through the brain of chicks.

Stability. The virus of rabies is preserved in

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glycerine at room temperature for several weeks, and at a temperature of 1 degree centigrade - for a year. The virus is preserved better and longer in a desiccated state. The loss of virulence depends principally on hydrolysis and oxidation, and, therefore, the virus should be stored in a desiccated state at low temperature in a vacuum. Fully protects the virus from destruction.

Under the effect of high temperature, the virus is rapidly destroyed. The viral suspension containing the first 2 cc of (2.5% formalin) for 1-2 days at 37 degrees centigrade. A water bath of 15 degrees centigrade inactivates with a suspension in 24 hours, at 50 degrees the inactivation takes one hour, at 62-65 degrees it takes 15 minutes, at 70 degrees - 5 minutes, and at 80 degrees - one minute - 20 seconds. The virus of rabies in vitro is highly susceptible to the effect of ultra violet light. A 5-10 minute irradiation is sufficient for the full inactivation of a concentrated virus suspension. Sunlight will destroy the virus in several minutes. The photodynamic effect of methylene blue inactivates the virus in 15 minutes.

A physiological solution and other isotonic solutions weaken the effectiveness of the virus. For the propagation of the virus, distilled water, with an addition of a 10 percent . . . serum (for example, rabbit serum), is best. The virus of rabies will disintegrate under the effect of ether in 120-

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the hours, under the effect of benzene - in 3 hours. The virus is inactivated by a one percent solution of formalin in 24 hours. One of the peculiar characteristics of the virus is its resistance to heat: a 10 percent solution of formalin at a temperature of 57 degrees centigrade will not inactivate the virus in two months. Alkali, nitrous, and nitric acids rapidly inactivate the virus of rabies.

Differentiation of the virus. The virus of rabies is distinguished from other neurotropic viruses, first of all, by the fact that it is usually pathogenic in a wide circle of animals. When, for instance, the diseased animal is a dog, the disease is not transferred to other animals. For serological differentiation, the neutralization test, i. e. neutralizing the tissue of virus with serum of an infected animal (produced on white mice), is used most frequently. For this purpose, an intra-cerebral inoculation of the mixture of the virus with amount sera, after keeping the mixture for one hour at 57 degrees centigrade, is used.

Epidemiology. Rabies is a disease known in all geographical latitudes. The source for the infection of humans are rabies-stricken wild and domestic animals. Most dangerous are the bites of rabies-stricken wolves, dogs, and cats; the latter cause small but deep injuries. Rabies are usually transmitted through the bites of dogs, which is corroborated by statistical data (90 percent of all cases). Cats are carriers in 4 percent of all cases. Rats and mice may also be carriers. The danger of rabies being transmitted by herbivorous animals is com-

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relatively small. There are cases on record, where rabies were transmitted through bats. It occurred in South America, on the island of Trinidad, and it caused tremendous losses in cattle and considerable mortality in humans.

Important factors are the location, the depth, and the nature of bites sustained, and also the amount of the bite-inoculated virus (the abundance or "concentration"). Most dangerous are head injuries and, partly blind, lacerations to mucous membranes.

There is no definite data on the existence of virus carriers or virus excretors (other than saliva), although there are indications on leaving the virus of rabies in their saliva, hair, and sweat, which were taken from stricken dogs.

Summary. The rabies is a fatal disease, cases of clinically identifiable rabies in humans are usually lethal. However, susceptibility of humans to rabies is relatively low.

Statistical data on the subject is very contradictory. In the sixties, only 25-35 percent of humans bitten by rabies-stricken animals and remaining unattended develop the disease. Cases of recovery from rabies in animals are very rare. It is, therefore, natural that no observations are available on the development of immunity by animals recovered from the affliction.

The creation of artificial immunity against rabies is attained by the subcutaneous inoculation of various anti-rabies vaccines. There are almost no direct indications as to the lasting effect of the immunity in humans after the prophylactic

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vaccination. It is assumed that immunity lasts for 12 months. This assumption is drawn analogically, by comparison with results of tests upon animals. A number of scientists point to the long lasting connection between immunity to rabies and the persistence of antibodies in the blood. However, to date, there is no exact answer to this question. It is also possible that not always is the non-susceptibility to rabies accompanied by the presence in the blood of the virus neutralizing antibodies. Also, the favorable results of anti-rabies vaccination in preventing the disease are not always accompanied by the appearance of antibodies in the blood.

To ascertain the dependence of immunity on the presence of antibodies, four virus injections, which revealed a very distinct infection, were given to mice. In the first group a live fixed virus and a virus (inactivated, 10⁶ per ml.) were both given, the second group showed non-susceptibility very early, on the fifth day after vaccination, and very retained the immunity for only a short time, 25 days. The number of antibodies, too, was increasing rather rapidly, attaining its maximum on the 10th - 15th day. The lowering of immunity was not in conformity with the titration standard of the antibodies in the blood.

There are indications to the effect that, in rabbits, immunity is connected not with the antibodies circulating with the blood, but with the antibodies in the cerebral tissue of the immunized animals. A supposition is also advanced that the mechanism of vaccination immunity in humans, who were bitten

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by rabies-stricken animals, can be explained by the interference as between the fixed and the street virus stems. The non-pathogenic (in humans) fixed virus of rabies penetrates into the central nervous system more readily than the virus of street rabies. The latter has a longer period of incubation (as testified by this report), and acts as the ^{antigen} ~~antigen~~ thus interfering the penetration by the virus of street rabies.

Classification and Therapy. For the time the current method of vaccination by the introduction of progressively increasing doses of desiccated rabbit brain, contaminated with the M. S. virus, is firmly established, a number of other prophylactic methods were submitted. They consist of the application of the virus, inactivated or not, either, subcutaneous, intracutaneous, intramuscular, or intracerebral, or the application of a live virus composed of various strains, such as repetitive freezing and thawing or desiccating.

The most popular vaccines in the world are: one prepared from a virus inactivated with alcohol (25 per cent), and another one prepared from fresh cerebral tissues mixed with glycerol (2.5 percent alcohol) (as per instructions). Of importance is also the local prophylaxis, which consists in the thorough decontamination of wounds in all cases of bites by suspect animals by burning out with a thermo-cauterizer, by cutting out [of tissues involved], by the use of disinfectants, such as mercuric chloride, protargol, collargol, and others.

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The effectiveness of anti-rabies vaccination is universally acknowledged. In the USSR, the existing network of veterinary stations, operated over the years of Soviet power, insured timely vaccination of animals. In the case of rabies is the more effective the earlier its application. Following is the description of the steps to be taken upon the suspicion of rabies in a dog, the observation of other rules of veterinary control. The prophylactic vaccination of dogs against rabies did not come into wide use.

Isolated animals. In certain circumstances it is necessary to isolate an animal as soon as the bite is sustained by a human. If a dog is involved, it must immediately be isolated and a decision made by the laboratory as to whether it is rabid or not. If after 10-15 days of observation, the dog involved remains healthy, its bite is considered as not dangerous. If the animal dies during this observation period, the presence of rabies in it is to be definitely ascertained. To date, no serological or other reactions, which could have been helpful in the diagnosis while the dog was alive, were developed as yet, and the laboratory decision on the diagnosis is based on the pathohistological examinations of the animal's brain, and on the result of inoculating rabbits or white mice with its cerebral tissues. The patho-histological examination brings out the presence in the cerebral tissue of typical

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microscopic examinations, i.e., the presence of hemi corpuscles in the brain neurons. The corpuscles are very characteristic, and they can be easily identified. They are always disposed in the cytoplasm of the cells, are easily stained, and they have a typical internal structure. Their presence is reliable as being an indication of the presence of the virus.

The most suitable laboratory animals for purposes of reliable diagnosis, in addition to rabbits, are now acknowledged to be mice also. The method for diagnosing rabies in mice since consists of confirming that the virus has been injected with a cerebral tissue suspension taken from the infected animal. In practice, however, the virus is injected into the brain tissue of the mouse following the inoculation, and they die within the subsequent 24 hours. The brain tissue from these mice contains hemi corpuscles.

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PART THREE

The Virus of Poliomyelitis (Infantile Paralysis)

General facts on the infection. Poliomyelitis is a grave infectious disease striking at human beings, especially children, and is frequently the cause for invalidism in children. The first clinical description of the disease was given by Selme. The first description of poliomyelitis as a disease (in Sweden, in 1835 and in 1935) was made by Selme. By the name of these two men, who were the first to give a clinically precise definition of the disease, poliomyelitis is often referred to as the Selme-Selme disease.

The clinical manifestations of poliomyelitis are exceptionally varied, and may be expressed by various symptoms of infection of the central nervous system, as well as by gastrointestinal disturbances, and other indications, little characteristic of poliomyelitis. The incubation period varies from 5 to 25 days, most frequently about 1-2 weeks.

The most typical manifestation of the disease is the development of atypical paralyzes of the upper and lower extremities, the muscles of the humeral belt and neck. Fever, headache, rigidity of the occipital muscles, and hyperesthesia frequently precede the appearance of paralyzes. The clinical diagnostics of the paralytical forms present no difficulties, but the clinical identification of atypical and abortive cases is a rather complex and difficult matter. Yet, ^{the} significance of the latter is very great, since in some epidemic outbreaks the number of abortive forms is 4-6 times the number of paralytic forms.

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the symptoms of abortive poliomyelitis are varied and non-characteristic, namely: short duration, fever of various intensity, and slight neurological symptoms. These general symptoms may be accompanied by headache, nausea, inflammation of the pharynx, diarrhea, sore throat, stiff neck. The abortive form is more frequently observed in older age groups. In acute poliomyelitis accompanied by paralysis, mortality is increased with age. Most frequently, death occurs during the first week.

A pathohistological examination of the brain of poliomyelitis victims exposes the disseminated character, along with the predominant lesion of the gray matter in the ventral horns of the spinal cord, with the close-active inflammatory infiltration of the pia mater. The most typical manifestations are the heavy losses of the pyramidal cells, particularly accentuated in the ventral horns of the spinal cord. Both diffuse vascular-inflammatory, as well as cellular-regenerative transformations are observed. Neurophagia and gliotic proliferation are sharply pronounced. Pathological transformations outside the central nervous system are most frequently discovered in the lymphatic tissue in the form of lymphoid hyperplasia.

Precipitation of the inciter. Hendriksen and Popper established in 1941-1952 that the disease is induced by a filterable virus, and can be transmitted to monkeys. At a somewhat later date a considerable amount of stems of the poliomyelitis virus was precipitated by way of intracerebral contamination of these animals with a cerebral suspension filtrate taken from the brain of patients who died from poliomyelitis.

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impaired spinal control between healthy animals and epileptic ones. The first phase of the histological examinations of the brain of epileptic animals showed the presence of transmutation, which was similar to that observed in the brain of humans (Figures 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100).

Figure 12. Experimental epileptic animals. The first phase of the histological examinations of the brain of epileptic animals showed the presence of transmutation, which was similar to that observed in the brain of humans (Figures 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100).

Figure 13. Experimental epileptic animals. The first phase of the histological examinations of the brain of epileptic animals showed the presence of transmutation, which was similar to that observed in the brain of humans (Figures 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100).

Figure 14. Experimental epileptic animals. The first phase of the histological examinations of the brain of epileptic animals showed the presence of transmutation, which was similar to that observed in the brain of humans (Figures 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100).

In 1939, Anderson succeeded in inoculating cotton-tail rats with the virus (Figures 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100). The virus was only precipitated from a brain and injected into another rat. After seven consecutive passages on rats, the virus was successfully inoculated into white mice.

Both the rats and the mice are susceptible to intracerebral contamination only. The incubation period, in the case of the

contaminated rats, is 3-5 days, in the case of mice, 1-30 days. The sickness is symtomized by paralysis of the extremities and the muscles of the back. The contamination of other species of rodents with the virus isolated to cotton-field rats was also successful.

Filterability, size, morphology. The virus of poliomyelitis is easily filterable: it passes freely through conventional filters, i. e. through -100-fold caniles, 0.4, 0.5, Chamber and 15 caniles. The size of the virus particles is determined by filtration through collection membranes. The virus is one of the smallest, its particles measure 1-1.5 millimicrons. The reports of some American scientists on the obtaining of two dimensions of the virus of poliomyelitis, on studying these with the aid of electronic microscopy, and on the discovery of isolated virus particles of peculiar morphology, cannot as yet be regarded as authentic, and they must be subjected to a thorough check.

Cultivation. The cultivation of the virus outside of a susceptible organism, irrespective of the many attempts, to date, has not been successful. Only sporadic data is available on the attempts at cultivating the virus in tissues, in the presence of neurons, such as fragments from the central nervous system of a 3-4 month old human embryo.

Stability. While being able to withstand low temperatures, the virus of poliomyelitis is very sensitive to high temperatures. Subjecting it to a temperature of 56 degrees centigrade for 30 minutes inactivates it completely. In a 50 percent solution of glycerine the virus can be preserved for years, in sterile water at room temperature - for 114 days. Under the same conditions, in

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Scandinavian countries. In the Soviet Union, it occurs
sporadically and, in rare cases, in the form of small
epidemic flares.

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Morbidity occurs in the cities as well as in rural localities, with the epidemic in rural districts running a heavier course. The epidemic flares show a tendency to be seasonal (summer-autumn), but sporadic cases may occur throughout the year. By age group distribution of morbidity, poliomyelitis justly merits the name of infantile paralysis. A great majority of all cases are children up to the age of five. In the USSR, by the Prisman data, 92.7 percent of all cases were children up to the age of 4, with most of the stricken being infants from 6 months to 2-year olds. Lethality in poliomyelitis, on the average, amounts to 10-14 percent, but is oscillating within wide limits, since the computations depend, to a considerable degree, on the inclusion into the statistics of identified abortive forms, which always have a favorable prognosis.

Accumulated voluminous data points to the fact that the gateway for the infection is the mucous membrane of the nose, the nerves of the olfactory system, and the alimentary canal, which plays a particularly important part. If we accept that the source of infection can be only a stricken person or a healthy virus carrier, it follows, then, that an important means for the dissemination of the virus is its precipitation through the intestines. The first discovery of the virus in the stool of patients and in the intestines, during an autopsy performed by Kling, has since been corroborated by numerous observations. In the stool of patients, the virus is found repeatedly after 2-3 weeks, and even a longer period after the recovery of the patient, it being the case that it is present in the stool of 70 percent of all patients. The pharynxes of the same patients were investigated, with the result that the virus was

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located in a smaller number of cases, and principally during the first days of the sickness.

It seems that, in the propagation of poliomyelitis, of decisive importance is the carrying of the virus in its abortive forms, which are numerous during epidemics. The virus was more than once segregated in atypical forms of the disease, in the stool of apparently healthy individuals, who were in contact with patients. The epidemiological significance of locating the virus in the stool of patients is corroborated by the fact that it is also found in the sewerage waters during epidemic outbreaks.

The concept of poliomyelitis as an infection, which is transmittable only by the drop method, has now undergone a change. The summer-autumn seasonability, the existence of the virus in the intestines and the discovery of it in sewerage waters, the possibility of transmitting the inciter through contaminated food products (milk), all these bring poliomyelitis epidemiologically close with the bacterial intestinal infection of typhoid. However, the theory of the drop method transmission of the infection cannot be altogether abandoned. It is based on the fact that the virus is precipitated from the pharynx of the patient, and frequently that monkeys can be experimentally infected by an intranasal inoculation.

Immunity. Poliomyelitis leaves in its wake a durable immunity. Cases of recurring sickness are very rare. The possibility of recurrence is due to the existence of antigenically different virus stems. Virus neutralizing antibodies are found

in the blood of humans who recovered from the disease. Numerous investigators proved that similar antibodies are encountered not only in the blood of reconvalescents, but also in the blood of healthy people, who were never stricken. The antibodies in the blood of normal people are encountered more frequently among the urban population (in 70-80 percent of the entire population), and not so frequently in the blood of the rural inhabitants. Newly born babies have antibodies, obviously obtained from their mothers. During the first 1-2 years of life, the number of antibodies gradually decreases, but subsequently appear again, attaining a maximum at about the age of 20.

In the mechanism of immunity to poliomyelitis, the antibodies in the blood do not play a substantial part. Persons having a large amount of antibodies in their blood can also be stricken. In poliomyelitis, immunity is, probably, stipulated not by the humoral, but by other, probably, local tissue factors. It was established that in the case of intra-cerebral inoculation of monkeys with the virus of poliomyelitis, antibodies, in high titration standard, can be discovered in the suspension prepared from the ventral cornus of the spinal cord. The monkeys were put to death on the 16th or 23rd day from the beginning of convalescence, when the antibody content in the blood was very low. Antibodies can be found in the cerebral tissue even 5 months after convalescence.

The virus neutralizing antibodies may be obtained by immunization of non-susceptible animals, such as horses and sheep.

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The activity characteristics of the sera obtained from them are not lower than those of the sera obtained from convalescents or monkeys recovered from the disease.

Following the discovery of the inciter of poliomyelitis, attempts were made to create artificial immunity by the use of a virus containing vaccine. These attempts were not successful. The live virus capable of inducing the formation of immunity was dangerous, since it was actually inducing the disease. A vaccine prepared from dead viruses was harmless but, as a rule, was poor in antigenic characteristics. Vaccines that gave good experimental results, when used on monkeys, turned out to be ineffective when used on humans.

Prophylactics and therapeutics. As yet, no practically valuable methods of prophylactics in the case of poliomyelitis exist. Various types of vaccines that were used for purposes of immunization of humans, were ineffective. The much publicized experiments with chemical prophylactics, intended to create a local carrier on the mucous membrane of the nose, which would hinder the penetration of the infection, also remained without results. Zinc sulfate, alum, picric acid were used for that purpose, but, with relation to humans, these local chemical-prophylactical methods remained ineffective.

Counter-epidemic measures to forestall the spread of poliomyelitis are based on the compulsory isolated hospitalization of the stricken, with thorough disinfection of their excretions, particularly the stool.

Special caution is to be exercised with regard to those who have been in contact with patients. Reports on the virus being discovered in drinking water and in the sewerage system are to be scrupulously checked, and strict caution in the use of drinking water, particularly in rural areas, to be exercised. It must be pointed out that the conventional chlorination dosages used in water purification are, seemingly, adequate for the inactivation of only small amounts of the virus. A chlorine concentration of 1.5 : 1000000 inactivates the virus in 20 minutes, a concentration of 0.55 : 1000000 in one hour.

For the last 30 years a serum from convalescents has been used for therapeutic purposes in poliomyelitis. However, the results of serotherapy are still debatable and contradictory. The majority of specialists consider the serotherapy ineffective. At best, the sera may be used with limited success in the pre-paralysis stage only, i.e. its use is rather prophylactic than therapeutic. However, there are some new developments in this field. There are reports to the effect that the gamma-globulin fraction of the serum from a human, i.e. the highly concentrated and free from inert albumins serum preparation may be therapeutically much more active than native sera.

Sulfamide preparations and many other chemico-therapeutical compounds were tested and found ineffective.

For the curing of cases of residual paralyses, specially developed methods of physical therapy and orthopedic techniques are utilized.

RESTRICTED

Laboratory diagnostics. The methods of laboratory diagnostics in poliomyelitis, regardless of voluminous experimentation, are still undeveloped. The complement fixation test, the precipitation test, the skin allergy test were tried many times, but found no practical application. The neutralization of the virus by sera from recovered or immunized humans and animals has only limited diagnostic significance, since it is utilized retrospectively. The presence of considerable numbers of healthy people with antibodies in their blood also reduces the diagnostic value of the neutralization reaction, particularly since, heretofore, it was tried only on monkeys, relatively expensive and rare animals. Now, with the availability of virus stems adapted to white mice, this procedure becomes more accessible and furnishes results analogous with those obtained on monkeys. The diagnosing of the disease by the direct precipitation of the virus from the stricken victim is very difficult, for, as a rule, the virus is not present in the blood and in the spino-cerebral fluid. The diagnosis can only be based on results of inoculating monkeys with a suspension from the stool or from the pharynx gargle of the patient. The posthumous virological diagnosis of poliomyelitis is effected by the intracerebral inoculation of monkeys with a finely ground suspension of nerve tissue fragments from the upper section of the spinal cord and from the medulla oblongata.

The Virus of Spring-Summer Tick Encephalitis.

General data on the infection. Spring-summer encephalitis is an acute and grave disease of the central nervous system, studied for the first time and segregated in 1937 into an independent nosological

RESTRICTED

RESTRICTED

unit by Soviet specialists (Zil'ber, Paner, Levkevich, Shubladze, Chumakov, Solov'yev).

The sickness develops after a 10-15 day incubation period and is characterized by a sudden start and tempestuous development of symptoms of the affection of the central nervous system. The first indicators are fever, headache, nausea, vomiting, hyperesthesia, which are then joined by local symptoms, most frequently paralyzes of the muscles of the neck, the humeral belt, and bulbar affections. The period of fever is 5-12 days long. The temperature oscillates between 38-40 degrees Centigrade.

During the acute stage of the disease, in the blood, there occurs leucocytosis, "lymphopenia", "eosinopenia", accelerated "ROE". There is some pleocytosis in the cerebrospinal fluid, an increase in albumen content, a somewhat heightened pressure.

Death occurs most frequently during the acute stage, between the first and the seventh day. Lethality does not exceed 30 percent. Stable residual phenomena, such as sluggish atrophic paralyzes of the muscles of the neck and the proximal sections of the upper humeral belt.

In patients who died from spring-summer encephalitis, a macroscopical examination shows edema, hyperemia of the brain and brain membranes, petechial hemorrhages in the cerebral tissue.

Pathohistological transmutations are characterized by the diffusivity and intensity of the inflammatory-degenerative process in the cerebrum and in the spinal cord. Particularly heavy lesions

- 207 - RESTRICTED

RESTRICTED

can be noticed in the brain stem, the medulla oblongata and the jugular-pectoral section of the spinal cord. This process involves not only the gray, but also the white matter. The inflammatory infiltrates are disposed along the run of the vessels and usually are diffusive or ganglionic in character. In the neurons there is a clear manifestation of degeneration, necrobiosis, and neuronophagia.

The precipitation of the inciter. The virus of spring-summer encephalitis was precipitated by Zlot'er, Levkovich, Shubladze, Chumakov, and Seier'fev in 1937 from the brain of humans who died from the disease. In all cases the precipitation of the virus was from the brain of a human who died not later than on the 7th day from the beginning of the disease. The virus precipitation tests were conducted via an intra-cerebral inoculation of white mice with a 10 percent suspension of cerebral tissue in a physiological solution. It is harder to isolate the virus from the blood, which incidentally is only possible during the first days of the disease. Even less reliable is the precipitation of the virus from the liquors.

Pathogenic characteristics to animals. Mice are highly susceptible to this disease. In intracerebral inoculations mice become infected with 0.03 cubic centimeters of virus in a 10^{-8} dilution. Another effective way of contamination is through the nasal chamber. Sickness can also be induced by introducing the virus subcutaneously, intradermally, onto a scarified cornea, intraabdominally, intravenously, or into the esophagus. But all

RESTRICTED

the above methods require a higher dosage of virus. The incubation period in mice is 4-5 days, but when small dosages of virus are used, incubation takes up to 14 days.

The first indication of the arrival of the disease is a sudden state of stimulation and accelerated sensitivity. With irritation, clonic convulsions frequently set in, usually ending in death or in the paralysis of dorsal extremities. The state of stimulation is changed into a state of delayed reaction, with subsequent paralysis. Death follows the same day.

Monkeys are susceptible to this virus, if inoculated intracerebrally or intranasally. The incubation period in monkeys is 5-9 days. The first indication of the sickness is fever and overstimulation. Then comes paresis, followed by paralysis. These symptoms disappear in convalescence, but sometimes stable paralysis remain, as in the case of humans. In monkeys, the disease runs its course in 7-10 days, and in 70 percent of all cases it ends in death.

Great numbers of various mammals and birds were tested for susceptibility to this virus. Positive results were obtained with wolf cubs, Amur hedgehogs, field mice, lemmings, evermann hamsters, domestic mice, siskins, "chechetkas", goldfinches, redpolls, chaffinches, sparrows.

Regardless of the methods of contamination used, the maximum amount of virus is discovered in the brain of the animal. Within 2 minutes after a subcutaneous injection the virus penetrates

RESTRICTED

RESTRICTED

into the central nervous system. Obviously, the virus penetrates into the brain by the hematogenic route.

When mice are contaminated intracerebrally, the virus can be discovered in the blood, lungs, liver, spleen, and kidneys within one hour. No propagation of the virus in the enumerated organs takes place, and the quantitative increase of the virus in those organs during the course of the disease is unquestionably a result of its propagation in the brain, from where it intensively passes into the bloodstream.

Stability of the virus. In cerebral fragments placed in 50 percent glycerine, the virus will retain viability for a year, if kept at a temperature of 4 degrees Centigrade, although its virulence is reduced considerably. A thick vacuum dried virus suspension can be maintained for 5 years. In a 10 percent suspension in a physiological solution, the virus will remain active for 15 days at 5 degrees Centigrade, for 10 days at room temperature (14-16 degrees Centigrade), for 2 days at 37 degrees. The virus has little stability to high temperature. The heating of a 10 percent suspension in a water bath at 60 degrees Centigrade will inactivate the virus within 10 minutes, at 70 degrees--within 5 minutes. Boiling kills the virus within 2 minutes.

Ultraviolet rays are lethal to the virus, which disintegrates in two minutes of irradiation.

Contact with alcohol, ether, and acetone destroys the virus within 3 days. The effect of a lysol solution upon the virus is as

RESTRICTED

RESTRICTED

follows: one percent lysol inactivates the virus in 5 minutes, 3 percent in 2 minutes, 5 percent in one minute. A one percent phenol solution kills the virus only in 10 days, 0.5 percent formalin in 48 hours. When in contact with methylene blue (1:25000), at a distance of 15 centimeters from a source of light in the form of a 100 watt incandescent lamp, the virus is destroyed in 15 minutes.

Filterability, sizes, morphology. The virus is filterable through Chamberland candles L₃, L₅, Berkefeld N, V, and Zeyts filters. The size of the virus, as determined by filtration through grade-perforation diaphragms, is 20-30 millimicrons. In studying the pathomorphological transmutations in the brain of stricken animals, Shen discovered in the cells of the cornu Ammonis acidophilous intranuclear inclusions, which are stained by the methods of Romanovsky-Giemsa, Mann and Unna.

Cultivability. Chumakov bred the virus in a tissue culture containing the pulverized organs of a chicken embryo suspended in a Tirole liquid with a 20 percent normal rabbit serum. The propagation of the virus in this culture was almost as intensive as in the intracerebral passages in mice. After 22 generations in the tissue culture, the virus retained its antigenic characteristics and was subject to neutralization by specific immune sera.

The virus was also successfully cultivated on the chorio-allantois of a 10-12-day old chicken embryo.

Differentiation of the virus. With relation to the similarity of the virus of spring-summer encephalitis with other neurotropic viruses, numerous investigations were conducted, and

RESTRICTED

RESTRICTED

differences established from the following viruses: rabies, poliomyelitis, Japanese encephalitis, encephalitis St. Louis, herpes, and others. However, it is more difficult to differentiate this virus from the virus of Scottish encephalitis, since there are cross-over immunity reactions as between the two viruses.

The analysis of the mutual relationship between the viruses of spring-summer encephalitis and Scottish encephalitis, made on the basis of serological reactions only, led to the supposition of identity between the two.

Tests by Zil'ber and Shubladze along the comparative study of the pathogenic characteristics of these two viruses to sheep, established an unmistakable difference between them. The clinical manifestations in sheep, contaminated with the virus of spring-summer encephalitis, were different from louping ill. The predominant symptoms in spring-summer encephalitis, that are absent in Scottish encephalitis, are pareses and paralyses. Histopathological transmutations in the brain of stricken lambs were also different as between the two viruses. For the louping ill virus, the lesions in the Purkinje's cells, occurring during the first days of the sickness, are characteristic, while in spring-summer encephalitis the Purkinje's cells are stricken alongside of other cells of the nerve tissue. In spring-summer encephalitis, intranuclear inclusions are discovered in the cells of the cornu Ammonis (Figure 45 which is a microphoto).

Figure 45. Experimental spring-summer encephalitis in a white mouse. Degeneration of the neurons in the

RESTRICTED

RESTRICTED

Cornu Ammonis. Intranuclear inclusions (Collection of the Institute of Virusology).

These intranuclear inclusions are absent in the case of Scottish encephalitis. The differentiation of the viruses is facilitated by the use of cottonfield rats, which are susceptible to the virus of Scottish encephalitis only.

The clinical symptoms in humans and (experimentally) in monkeys are also sharply different as between the two viruses.

Epidemiology. There is a clear seasonal characteristic in spring-summer encephalitis. The virus strikes beginning with the first 10 days in May, attaining its maximum toward the beginning of June. In July and August only sporadic cases are registered. Another essential characteristic of this virus is that it is endemic. The endemic centers of spring-summer encephalitis usually lie in a mixed broad-leaved and coniferous forest in a moderate temperature zone. In addition to the Far East, endemic centers are now known to exist in the Urals, on the Volga, and in some regions of the West European part of the USSR. The virus strikes more frequently at persons who are professionally connected with forestry maintenance and service. The most susceptible age ranges from 20 to 40 years. Persons freshly arrived into an endemic zone are more liable to be stricken. Local inhabitants are considerably less liable to the disease.

Based on the epidemiological characteristics of spring-summer encephalitis, a theory was advanced with relation to the possibility of the existence of a carrier. Observations by Pavlovskiy, .

RESTRICTED

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Zil'ber and their collaborators brought out the connection between morbidity in humans and the seasonal dynamics of ticks. The significance of ticks as carriers of spring-summer encephalitis was proven by direct laboratory tests. In 1937, Chumakov experimented with mice and established the possibility of the virus being carried by *Ixodes persulcatus* ticks. Ryzhov and Skzynyuk, by feeding ticks collected in the "layka" [Northern forests] on the blood of mice, precipitated a virus that turned out to be identical to the inciter of spring-summer encephalitis.

Out of a quantity of *Ixodes persulcatus* ticks, which were collected in various endemic centers, Shuoladze and Serdyukov precipitated 28 virus stems. The precipitation of the virus was effected by the method of intra-cerebral inoculation of mice with a suspension of triturated ticks and by allowing the ticks to feed on the blood of living mice.

Subsequently, the possibility of the durable survival of the virus in the organism of the ticks and its trans-ovarial transmission were established. The durable survival of the virus in the ticks and its transmission to their progeny point to the fact that the ticks are not only the carriers of the virus but also its reservoir. Since the life of ticks is tied in with their parasitism on warm-blooded animals, the possibility of some vertebrates serving as the natural virus reservoir loomed up. As per above, a series of species of wild animals and birds are susceptible to contamination. In addition, investigations by Solon'yev established that some wild animals are virus carriers. Virus stems were precipitated from field mice, moles, hedgehogs. This shows that, in the endemic centers of

RESTRICTED

RESTRICTED

spring-summer encephalitis, not only ticks, but also some mammals are virus carriers. The virus of encephalitis circulates from the contaminated tick to a mammal, and from the latter, in turn, new contingents of ticks are contaminated. Thus, a closed cycle of virus circulation stipulates the natural endemic character of the disease. (Pavlovskiy)

Immunity. No recurrence of the disease was registered. Beginning with the 15th day of the sickness, antibodies appear, gradually increasing in number and attaining its maximum in 2-2½ months. The antibodies are preserved for years, and can be discovered after 12 and even 25 years.

In the blood of the inhabitants of the endemic zones, antibodies may appear not only as a result of a clinically pronounced sickness, but also as a result of symptomless infection or an imperceptible immunization, which is tied in with having been stung by a virus carrying tick. The presence of antibodies in the blood of the inhabitants of endemic zones points to the presence of immunity. This explains the low rate of morbidity among the inhabitants and the higher rate of same among the newcomers.

The relation between the presence in the blood of antibodies and non-susceptibility to the disease was established experimentally by way of laboratory tests on animals.

Immunized animals with a large count of antibodies in their blood proved to be non-susceptible even to intracerebral inoculation with the resistivity to contamination increasing parallel with the titration standard of the antibodies.

RESTRICTED

Prophylactics and therapy. Prophylactic measures in spring-summer encephalitis are conducted along two lines: (1) combatting the ticks, and (2) immunizing the populace with vaccine.

The vaccine against spring-summer encephalitis, which was first suggested by Kogan, Smorodintser, and Lerkovich, is a 5 percent suspension of cerebral tissue from contaminated mice, in which the virus is inactivated by 0.1 percent formalin and 0.25 percent phenol solutions. A subcutaneous double inoculation with 3 and 5 cubic centimeters of the vaccine, lowers morbidity considerably, moderates the course of the disease, and reduces mortality. In individual cases, with diagnosis established, seroprophylactics are used. The serum is to be inoculated intramuscularly 2 or 3 times at 5-6 day intervals in the amount of 20-30 cubic centimeters.

Best results were obtained by the use of a serum from convalescents. Introduced endolumbarly in dosages of 10-15 cubic centimeters, the serum reduces fever rapidly and improves the general condition of the patient. For the success of this method, it is important to use the serum prior to the third day of the sickness.

Laboratory diagnostics. The laboratory diagnostics of spring-summer encephalitis consists of the precipitation of the inciter and the discovery of antibodies. For the precipitation of the virus from the blood, the most favorable period, as was already pointed out, is the first week of the disease, at the height of its fever stage.

RESTRICTED

In case of death, the brain and the internal organs serve as the material for the precipitation of the virus. Experience shows that the virus is detected with maximum regularity and ease in the brain of patients who died not later than on the 7th day from the beginning of the disease. The detection of the virus in such internal organs as the liver and spleen, and, also, in the body liquors and urine, is not regular, and, therefore, cannot be used diagnostically.

Antibodies in the blood of patients recovered from the sickness are detected with the aid of the complement fixation test and the neutralization test. The complement fixating antibodies can be detected as early as the first two weeks. The neutralizing antibodies appear later and are accumulated in large numbers after 2-2½ months from the beginning of the sickness.

The Virus of Japanese Encephalitis

General data on the infection. Japanese encephalitis is a grave infectious disease of the central nervous system.

The clinical symptoms are extremely sharp, and are expressed by general toxico-infectious phenomena in the presence of meningeal and general cerebral symptoms. The beginning of the sickness is usually manifested by a sudden attack of high fever, sharp symptoms of irritation in the cerebral membranes and the brain itself, accompanied by delirium, frequent loss of consciousness, convulsions, intense headaches, nausea, vomiting, and occipital rigidity. Paralytical symptoms are also observed, most frequently of the hemiplegia type, with a trend toward reduction.

- 217 RESTRICTED

RESTRICTED

Frequently, bulbar disturbances of the acts of respiration, deglutition, and speech occur. There are almost no sensory disorders. Psychic disturbances are frequently registered. The most typical transmutation in the blood is leucocytosis, the intensity of which runs parallel with the gravity of the sickness (average figures being 11800-18700).

With an unfavorable course, death usually occurs on the 5th or 6th day of the sickness. Mortality increases with age. For the age of 11-20 years it is 40 percent, for the age of 70-80 years it is 83.7 percent, for the age of over 80 years it is 92.9 percent. Average mortality is 60-70 percent.

In the cases of recovery, there is rapid disappearance of the symptoms, there is rapid disappearance of the symptoms, most frequently without any residual nuclear phenomena left.

Macroscopic transmutations in the brain of patients who died consist of hyperemia and edema of the cerebral membranes, sometimes insignificant hemorrhages into the brain. Microscopic transmutations are expressed in the form of general and nuclear progressive transmutations of the glia tissue with the formation of glionic ganglia and the predominance of lymphocytes. Degenerative transmutations of various intensities, with neurophagia, occur in the neurons. Perivascular infiltrates and connections consist mainly of circular cells and monocytes. All transmutations are predominantly in the gray matter of the brain and the spinal cord.

Precipitation of the virus. In studying the etiology of

- 218 - RESTRICTED

the disease, Japanese scientists fell into a series of methodological errors, and the results arrived at by various authors, were frequently of a mutually contradictory nature. Only in 1933, Khayasni succeeded in reproducing in monkeys a clinically pronounced case of Japanese encephalitis by inoculating it with the cerebral tissue of a human who died from this disease. Then, from the brain of a stricken animal, by way of contaminating mice, the virus was precipitated. Etiological investigations of Japanese encephalitis began on a large scale after the Soviet scientists Shubinauze, Smorodintser, Neustroyer, in 1938 precipitated several dozens of virus stems of Japanese encephalitis from the blood, body liquors, and urine of patients, and from the brain of victims who died during the outbreak of the disease over the territory of the Soviet Maritime Provinces. The virus is successfully precipitated in the first 7 days of the sickness.

Pathogenic character with relation to animals. Mice are the most susceptible to intracerebral contamination, but they can also be infected by a subcutaneous, intravenous, intraabdominal, intranasal, and intratesticular inoculation. The clinical symptoms in mice run in two forms--paralytic and lethargic. In the paralytic form, 4-7 days after incubation, the sickness is manifested by tremor, spasms, paralyzes, prostration. Death comes within 24 hours. In the lethargic form, a general tremor of the entire body is present, together with little mobility. Death comes within 24 hours. In investigating the distribution of the virus in the organism of contaminated mice, the maximum numbers were found in the brain and in the spinal cord.

Monkeys are also susceptible to Japanese encephalitis. The clinical symptoms in monkeys and in humans are similar.

In addition to mice and monkeys, the following animals and birds are susceptible to experimental contamination: sheep, female goats, infant mice, hamsters, eastern field mice, "chechetki", goldfinches, stickins, and redpolls.

Pathologico-anatomical transmutations in experimentally contaminated animals are similar to those observed in humans. Mainly the gray matter of the brain is affected. The repletion of the circulatory vessels of the brain, peri-vascular infiltrates with a predominance of large cells and monocytes--are sharply pronounced. There occur general hyperplasia, grave degenerative transmutations in the neurons, necrosis and neuronophagia nuclei (Figure 47, which is a microphoto).

Figure 47. Experimental Japanese encephalitis in a white mouse. Inflammatory reaction around circulatory vessels of the subcortex zone. Stained by the method of Romanovskiy-Giemsa (collection of the Institute of Virusology).

Rabbits, guinea pigs, rats, dogs, cats, pigeons, and chickens are not susceptible to this virus. However, in guinea pigs, during intratesticular passages, the virus survives in the ova, and can be discovered by inoculating white mice with the infected tissue.

Filterability, size, morphology. The virus is filterable through Berkefel candles V, N. W, Chamberland L₂, L₃, and through

RESTRICTED

Zeyts filter. The size of the virus particles is 20-30 millimicrons.

Cultivation. The virus can be successfully cultivated in vitro on pulverized tissues of a chicken embryo, which are suspended in allantois fluid or the Tirode fluid. The growing chicken embryo is a favorable medium for the propagation of the virus. It was established that the best method of contamination is by inoculation into the yolk sac of 7-8 day embryos with a subsequent 48 hour incubation at 36-37 degrees Centigrade.

Stability. The virus is best preserved in a 50 percent glycerine solution at low temperatures, after having been desiccated in a vacuum. When the virus is stored in the form of a 10 percent cerebral tissue suspension in physiological solution at room temperature (14-15 degrees Centigrade), it becomes considerably inactivated in 10 days. The complete inactivation of the virus takes place at 37 degrees Centigrade in 2 days, at 56 degrees in 30 minutes, at 70 degrees in 10 minutes, at 100 degrees in 2 minutes. The optimum concentration of hydrogen ions for the survival of the virus is 7.4.

Acetone, alcohol, and ether kill the virus in 3 days. The inactivating effect of lysol is particularly pronounced: One percent lysol will inactivate the virus in 5 minutes, 3 percent in 2 minutes, 5 percent in one minute.

Differentiation from other viruses. By its biological characteristics and pathologico-anatomical inroads, the virus of Japanese encephalitis is very similar to encephalitis St. Louis. However, the serum from convalescents and the serum from virus-

RESTRICTED

immunized animals are capable of neutralizing the virus of Japanese encephalitis in higher concentrations, while the virus St. Louis is either neutralized by these sera to a mild degree only, or not at all. The virus of Japanese encephalitis is pathogenic to mice under any method of inoculation, while a subcutaneous inoculation with the virus of St. Louis has no positive results. Monkeys are considerably less susceptible to the virus of St. Louis than to the virus of Japanese encephalitis. Contamination takes effect only when massive doses are inoculated intracerebrally, and in 50 percent of all stricken monkeys occurs a non-lethal mild form of encephalitis. Sheep are susceptible to Japanese encephalitis only. Peculiar for the pathohistological transmutations in Japanese encephalitis, is the presence of nuclei of the softening of nerve tissue.

Epidemiology. Epidemics of encephalitis were observed in Japan since 1904, but up to 1924, the registration of the sick was irregular and sporadic. After the great epidemic of 1924, the sickness was segregated into a class by itself. In differentiation from encephalitis Economus which was called encephalitis type A, Japanese encephalitis was called encephalitis type B.

The endemic centers of encephalitis are distinguished by their stability. The maximum number of cases occur in rural areas, but the sickness is also known in the cities.

The strongest epidemiological characteristic of Japanese encephalitis is its summer seasonability, due to which the sickness

is frequently referred to as summer encephalitis.

As a rule, the outbreaks occur at the end of the summer and end with the arrival of cold weather. The peak of morbidity is reached in August and September. The following mosquitoes are the virus carriers: *Culex tritaeniorhynchus*, *Culex bitaeniorhynchus*, *Culex pipiens*, *Aedes excrucians*, *Aedes togoi*, *Aedes japonicus*.

In the endemic centers, the virus carrying on the part of healthy people, animals, and birds was established. Keeping in mind that mosquitoes, such as *Culex pipiens*, are frequently parasitic on birds, and that some birds of the sparrow group are susceptible to the virus, the possibility suggests itself that birds serve as the natural reservoir of the virus of Japanese encephalitis.

The possibility is not excluded that rodents, as well as other species of animals and birds, which are constantly being attacked by virus-carrying mosquitoes, serve as an important link in the natural circulation of the virus.

Immunity. Japanese encephalitis, in cases of recovery, leaves in its wake a stable immunity, and recurrent attacks are rare.

Specific virus neutralizing antibodies appear in the blood of convalescents beginning with the 8-10th day of the sickness. They can still be found in the blood after months and even years. They are also found in the blood of healthy people located in the epidemiological zones, which fact testifies to the existence of abortive, or subclinical, forms of the infection. This is

corroborated by experimental data. Animals that recovered from encephalitis, or were inoculated with it without developing any symptoms, a phenomenon occurring rather frequently in monkeys, turn out to be immune to the subsequent contamination and show the presence of antibodies in their serum and blood. In immunized animals, stability to repetitive contamination is in direct ratio to the presence of antibodies in their blood.

Prophylaxis and therapy. Among the preventive measures of primary importance are those which protect humans from attack by mosquitoes--the carriers of the virus.

Alongside of these general counter-measures, there is a specific prophylaxis method of immunization with a formalized vaccine prepared from the brain of contaminated mice.

The therapy also calls for the use of a specific immune serum.

Laboratory diagnostics. Of considerable help in the clinical diagnostics of the infection is the complement fixation test, since the complement fixating antibodies appear as early as the 4-5th day of the sickness. The virus neutralizing antibodies appear in adequate numbers at a considerably later date. This is why the neutralization test is used only for retrospective diagnosis. It is best to take blood for the above test not before a month from the beginning of the disease.

Another important fact in confirmation of the diagnosis in Japanese encephalitis is the precipitation of the virus from the

blood and body liquor of patients and from the brain of those who died from the infection. Positive results are at their maximum during the first seven days of the disease.

The Virus of Encephalitis St. Louis, or American Encephalitis.

General data. Encephalitis St. Louis became known as such after the epidemic outbreak of 1933 in the city of St. Louis, the United States of America.

The clinical symptoms develop after an incubation period of 4-21 days, and most frequently begin with a headache and high fever, followed by occipital rigidity, tremor, frequent loss of consciousness, psychic disturbances, and rapidly passing perturbations of vision. The fever reaction is not characteristic. The acute period with the maximum rise in temperature lasts, on the average, 5-7 days. No particular changes occur in the blood and body liquors. Mortality rises with age. Up to the age of 40 it does not exceed 3-5 percent; in the 40-60-year group it is 20-25 percent; in the age group above 60 it attains 50-60 percent.

Residual phenomena are observed in 4.6 percent of all cases of convalescence. These consist of intellectual dullness, general weakness, emotional instability. Frequently, after the acute phase of the sickness, there are paralyses and weakness of the facial muscles, easy exhaustibility, vertigo, headaches, tremor in the extremities.

Histopathological examinations of the brain and spinal cord discover the dilation of circulatory vessels, hemorrhages, the formation of perivascular infiltrates. Frequently there are nuclear

RESTRICTED

accumulations of inflammatory cells in the cortex, the mesencephalon, the medulla oblongata and the pons Varolii, also degeneration of cells. No specific transmutations in other organs are noticeable.

Precipitation of the virus. The virus was precipitated in 1933 by Mockenfus, Armstrong, and MacCordick by way of contaminating monkeys, and by Webster and Fyfe by way of contaminating white mice. In studying the precipitated stems at various times and in various areas, it was established that they are identical and always pathogenic to monkeys and white mice. Subsequently, the virus stems were also precipitated during the outbreak of 1937 in St. Louis, and later on.

Pathogenic characteristics. Mice and monkeys are the most susceptible to this virus. The intracerebral inoculation of mice with the virus of St. Louis bring on, after 3-4 days of incubation, tremor, convulsions, ataxia, paralysis, prostration. The animal perishes in 5-9 days after having been contaminated. The virus propagates rapidly in the brain of these animals and attains high concentrations. Intranasal contamination in mice also induces the infection in 4-5 days, with the presence of the virus in the olfactory tract discoverable after 24 hours, in the brain--after 3 days, and in the spinal cord--after 4 days. Subcutaneous or intraabdominal inoculations do not produce the disease in mice.

Monkeys are susceptible to the intracerebral inoculation with this virus, with the sickness accompanied by high feverishness, high temperature, apathy, tremor, paresis. However, subsequent passages of the virus in monkeys cause the dilution of the virus, and for the

preservation of the virus stems, passages on mice are employed. Pathomorphological transmutations in the brain of animals that perished are similar to those observed in humans.

Rabbits, guinea pigs, rats, sheep, cats, and skunks are not susceptible to the virus of St. Louis.

Filterability, size. The virus is filterable through Berkenfeld candles V, N. W, and Zeyte filters. The size of the virus is determined by filtration through guado-perforation diaphragms, and it equals 22-23 millimicrons.

Cultivation. The virus of encephalitis St. Louis is cultivable in a medium consisting of a Tirode solution containing the pulverized brain of a mouse embryo. It is also cultivable on the chorio-allantois of 12-16-day old chicken embryos, causing necrotic transmutations on the allantoic membrane, which transmutations are manifested with the use of vital staining.

Stability. During intervals between passages, the virus survives for no less than 2 months in 50 percent glycerine in a cooler at 4 degrees Centigrade. In the form of a 10 percent brain suspension from stricken mice, at minus 70 degrees Centigrade, after having been vacuum-dried, it survives for many months. At a temperature of 56 degrees Centigrade, the virus disintegrates in 30 minutes.

The virus becomes inactivated in 10-18 hours, at room temperature, by the addition of 0.1 percent of formalin, and in 50 days by the addition of one percent phenol.

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The differentiation of the virus. The virus of encephalitis St. Louis is closely related to the virus of Japanese encephalitis. However, differences were established. Monkeys are less susceptible to the virus St. Louis than to the Japanese virus. White mice become infected with the virus of Japanese encephalitis through any method of inoculation, while an effective contamination with the virus St. Louis can be attained by two methods only: intracerebral and intranasal contamination.

The serum of animals (monkeys, rabbits, mice), immunized with the virus of Japanese encephalitis, alongside the active neutralization of the homologous virus, is also capable of neutralizing the virus St. Louis, but only to a milder degree.

In order to differentiate these two viruses, the method of vital staining of the transmutated allantoic membrane is used. It was established that the virus St. Louis can be neutralized with a specific immune serum, by using a mixture of immune serum and the virus to contaminate the chorioallantois of a chicken embryo. Eggs contaminated with such a mixture were compared with standard eggs, which were contaminated with a mixture of virus and serum, specific for Japanese encephalitis, a mixture of virus with normal serum, or with eggs, contaminated by the virus only. The complete absence of nuclei of transmutations or a perceptible reduction in their number, as compared to standard, proved specific neutralization. Staining with methylene blue, while the animal is still alive, facilitates the identification of virus nuclei. In these experiments, complete neutralization of the virus of encephalitis St. Louis, by specific immune rabbit sera and sera of convalescents, was effected,

while no crossover reaction was observed with the virus of Japanese encephalitis.

Epidemiology. A clear epidemiological characteristic of encephalitis St. Louis is its seasonal occurrence during the summer.

The avenues of propagation for encephalitis St. Louis were not ascertained until 1942. In 1942, data published by Hammon and his collaborators furnished essential data relating to the significance of mosquitoes in the propagation of this infection.

The authors cite data on the widespread parasitism of the mosquitoes *Culex tarsalis* upon various species of animals, such as horses, cows, pigs, dogs, chickens, and, also, humans. *Culex tarsalis* are capable of hibernating in its adult stage, and it is possible that the virus in them survives. In 1940, a report was published to the effect that the reservoir of the virus are ticks--*Dermanisus gallinae*, which are parasitic on birds.

When domestic and wild animals, inhabiting the endemic areas, were investigated during the tests for the neutralization of sera, it was established that from 35 to 50 percent of them had antibodies in their blood.

Immunity. There are no known recurrences of this disease in the same individual. Immunological research has shown that during the height of the sickness, as well as during the convalescent period, the patients accumulate specific antibodies in their blood. The amount of antibodies in the serum of patients recovered from the sickness reaches its maximum after 1 - 1½ months, and it is retained

RESTRICTED

In their blood for 2½ years, after which period their titration standard gradually diminishes. It is possible that the frequent discovery of neutralizing antibodies in the blood of healthy people, who live in the endemic centers, is a result of an unmanifested sub-clinical form of the infection. In the experimental investigation of immunity in mice, it was discovered that the antibodies appear prior to the resistivity to intracerebral contamination, and they disappear with the continued increase in resistivity.

Prophylaxis and therapy. After the discovery of the carrier of encephalitis St. Louis, the possibility was opened for the organization of prophylactic measures directed against the mosquito vectors. There is no specific therapy for this sickness, and it is conducted by analogy with similar virus diseases, proceeding mainly symptomatically.

Laboratory diagnostics. The laboratory diagnostics of the disease has no peculiarities of its own. It is similar to the above described procedure in Japanese and tick encephalitis, and consists in the precipitation of the virus and the detection of antibodies by way of neutralization and complement fixation tests.

The Virus of Scott's Encephalomyelitis of Sheep (looping ill).

General data. The sickness first became known in Scotland and in the north of England about a century ago. This virus is pathogenic to humans. Cases of [accidental] laboratory contamination of humans are on record. The sickness was characterized by headache and fever. Other symptoms were: somnolence changing to insomnia, nausea,

RESTRICTED

vomiting, double vision, disturbance of reflexes, and general weakness. During the acute period there was some leucocytosis in the blood, and in the cerebrospinal fluid there was an increase in globulin and mononuclear pleocytosis.

Precipitation of the virus. Meffe was the first to establish in 1931 that the filter of encephalomyelitis in sheep was a filterable virus. He proved the infectious characteristics of a filtrate prepared from the brain of stricken sheep. This was subsequently corroborated by a series of other scientists.

In 1934, Zil'ber and Shkoladze indicated that the virus precipitated by Gromakov from ticks *Ixodes ricinus* in Belorussia, resembles to a high degree the virus of Scottish encephalomyelitis of sheep. Subsequently, Zil'ber and Zacharov precipitated from ticks a series of new strains, which were studied and identified as the virus of Scottish encephalomyelitis of sheep.

Pathogenic properties. Sheep are highly susceptible to the virus of encephalomyelitis. They are contaminated through the inoculation of the virus into the brain and spinal cord, into the sciatic nerve, the nose, the conjunctiva. A subcutaneous inoculation and an intravenous inoculation frequently cause an abortive form of the sickness accompanied by the mere development of fever. Contamination through the mouth is not successful. In sheep, the disease strikes at the central nervous system and manifests itself in high fever, convulsions, tremor of the head and other parts of the body, continuous moving about in a circle, precipitately and by leaps. Specifically this symptom led to the name "avertin" or "snaker" of sheep.

RESTRICTED

Mortality among sheep is very high. There are also chronic and abortive forms of the disease.

Mice are susceptible to the intracerebral, intraperitoneal, subcutaneous, and intranasal inoculation. The symptoms are expressed in a state of agitation, restlessness, waverine motion, paralysis, with death occurring on the 4th - 5th day. *Macacus rhesus* monkeys are susceptible to intracerebral contamination. After 4 - 5 days of incubation, the animal's temperature rises to 39 - 40 degrees Centigrade, it becomes passive, loses its appetite; there is incoordinated motion (ataxia), as a result of which the animal, moving about the cage, frequently falls.

Other animals susceptible to this virus are calves, pigs, and field mice. In 1940, the author established the susceptibility of cottonfield rats, and the possibility of adapting the virus to white rats.

The predominant pathological transmutations in animals are meningeal and perivascular infiltration, disintegration of the Purkinje cells in the cerebellum. Mononuclear cells predominate in the infiltrates, in the diffusion and nuclear cellular reactions, microglia cells prevail. Intracerebral inclusions in the brain neurons of mice are not described.

Filterability and size. The virus passes through Chamberland 12, 13 and Berkefeld W, V, N filters. The size of the virus, as determined by filtration through a colloidal diaphragm, is 15 - 20 millimicrons.

- 232 -

RESTRICTED

RESTRICTED

Cultivation. The virus is cultivated in a medium containing fragments of chicken embryos in Tirode liquid. The most successful cultivation of the virus takes place in a developing chicken embryo. For this purpose, a contamination of the chorioallantois of a 12-day chicken embryo is effected. After 3 days of incubation, small white foci, 0.2 - 0.3 millimeters in diameter appear. They are then surrounded by still smaller foci. The titration standard of the virus on the chorioallantois attains the value of 10^{-7} .

Stability. In a desiccated state the virus can survive for several years, in 50 percent glycerol -- up to 6 months. The virus is unstable to temperature effects: a hot water bath of 50 degrees Centigrade will inactivate it in 10 minutes. A 1 - 5 percent solution of caustic soda kills the virus within one minute. Alkali solutions of higher concentration will coagulate the suspension and will not penetrate deeply into the latter without thorough agitation, as a result of which the virus will remain virulent inside the coagulate. A 1 - 6 percent phenol solution will not kill the virus in 5 minutes.

Differentiation. The virus of Scottish encephalomyelitis is closest of all to the virus of spring-summer encephalitis. The serological differentiation of these viruses is difficult, but the experimental contamination of sheep with both these viruses, the study of the clinical symptoms and the pathomorphological transmutations of each were instrumental in the differentiation of these two viruses. More details were furnished in the chapter on spring-summer encephalitis.

RESTRICTED

RESTRICTED

Epidemiology. Spring seasonality, coinciding in time with the maximum propagation of the ticks *Ixodes ricinus*, which are the virus carriers, is characteristic. Zil'ber and his collaborators indicate the presence of Scottish encephalomyelitis of sheep in the USSR. They succeeded in precipitating several strains of the virus from stricken sheep and from naturally collected ticks *Ixodes ricinus*. Glazunov and Popova discovered several cases of the disease in humans, established by clinical and virological diagnoses as Scottish encephalitis.

Immunity, prophylaxis, therapy. The experimental study of immunity established the relations between resistance to the disease and the presence of antibodies in the blood. Stable immunity can be attained by the inoculation of the active as well as the inactivated virus. Ultraviolet vaccine is suggested, since its immunogenic characteristics are higher than those of formalized vaccines.

Laboratory diagnostics consists of precipitating the virus, its subsequent identification and the detection of antibodies.

Virus of Encephalomyelitis of Horses.

General data. Encephalomyelitis of horses is an acute infectious disease of the central nervous system, which is induced by a virus pathogenic also to humans. Epidemic outbreaks of this sickness among humans are on record only in the United States. No other country reports any epidemic or sporadic contamination of humans.

RESTRICTED

It is now established that there are several varieties of horse encephalomyelitis. Three such varieties, designated as the eastern and western type, and the Venezuela virus, are present in America. In Germany and France the sickness is known under the name of the horn disease. The presence of the horn disease as well as other varieties of encephalomyelitis in horses has been established in the USSR.

The sickness in humans is manifested clinically by a sudden start, headache, nausea, fever, and lethargy.

The predominant neurological symptoms are muscular weakness and hyperreflexia. In the blood there is leucocytosis with an increase in polymorphonuclear neutrophils. The cerebrospinal fluid flows out under increased pressure, and it contains from 200 to 500 cellular elements within one cubic centimeter. Albumen content is increased. The duration of the sickness is 1 - 2 weeks, mortality is 10 percent. In children, the sickness leaves in its wake residual phenomena: periodic convulsions and intellectual sluggishness. Chronic forms of the disease are also described.

Examinations under the microscope show edema and hyperemia of the circulatory vessels of the brain and the spinal cord. Micro-transmutations are characterized by inflammatory phenomena in the brain membranes and substance, particularly in the area of the basal cores. In individual cases, small disseminated nuclei of demyelination are observed.

Precipitation of the inciter. In studying the epizootic outbreaks of encephalomyelitis among horses, which have been

- 235 -
RESTRICTED

RESTRICTED

observed for tens of years in the United States, Meyer, Goering and Gouvit precipitated the virus from the cerebral tissue of stricken horses. Subsequently, virus stems were precipitated in various American areas.

Several virus stems were precipitated by Soviet investigators from the brain of stricken horses. Vysnelovski, was the first to precipitate the virus stem at Alma-Ata. By now, several tens of virus stems of horse encephalomyelitis are recorded in the USSR. Available data permits the deduction that these viruses, by their characteristics, differ from the American variety and the inciter of the form disease, and can, in their turn, be divided into several types, differentiated by various degrees of pathogenic characteristics with relation to horses.

Pathogenic properties. The virus of American encephalomyelitis of horses is pathogenic to the most diverse species of animals, and in this respect it can be compared with the virus of rabies. Among the laboratory animals susceptible to this virus are white mice, rats, guinea pigs, rabbits, monkeys. Mice can be successfully contaminated by any of these methods: intracerebral, intranasal, subcutaneous, intravenous, and others. The incubation period for the western variety of virus is 4 days, for the eastern variety -- 40 hours. Susceptibility to extraneural contamination with the western and eastern variety of the virus decreases with the age of the mice. Twelve - sixteen-day old mice are almost as sensitive to extraneural contamination as they are to intracerebral, and for adult mice the dosage in peripheral contamination has to be greatly increased. The susceptibility to the various methods of inoculation

- 236 RESTRICTED

RESTRICTED

with the Venezuela virus does not vary with age. In addition to laboratory animals, female goats, sheep, calves, pigs, cottonfield rats, and many species of birds are susceptible to this virus.

The fatherland virus stems are considerably diversified by their pathogenic qualities. The Alma-Ata stem, in addition to horses, strikes only at cats. Other stems are pathogenic to horses and rabbits. Yet, there are stems which, like the American virus, will strike at various animals. Such a one is the Leningrad stem. In studying the susceptibility to the virus of various birds, it was established that chicks, goldfinches, and greenfinches are susceptible.

The virus of horse encephalomyelitis (excepting the born disease) is not strictly neurotropic. While the highest concentration of the virus in stricken animals is found in the brain, it is also present in the spleen, regularly during the fever period, and less regularly during the incubation period. Histological examinations in the brain of the stricken animals are characterized by the presence of diffused inflammatory and perivascular masses and degenerative changes of cells, particularly in the cornu Ammonis.

Filterability, sizes, morphology. The virus of American encephalomyelitis easily passes through Zeitz and Berk field filters. By the method of ultrafiltration, the size of the virus was determined to be 20 - 30 millimicrons. In studying the virus with the aid of an electronic microscope, it was discovered that the particles are spherical with a diameter of about 22 millimicrons for the eastern variety and 39 millimicrons for the western variety.

RESTRICTED

RESTRICTED

The fatherland virus stems are somewhat larger in size -- between 85 and 130 millimicrons.

Cultivation. All virus stems of the American horse encephalomyelitis can be successfully cultivated on the chorio-allantois of a chicken embryo. Best results are to be had with 10 - 11 day old embryos. The death of the embryo occurs after 15 - 24 hours. All the viscous of the embryo contain the virus in practically the same concentration, still the maximum titration standard can be noted for the embryo proper and the chorioallantois. Experiments with the cultivation of fatherland virus stems on chicken embryos were conducted by many researchers, and they produced positive results.

Stability. In a desiccated state, the virus will survive several years, and in a 50 percent glycerin solution, with the pH = 7.4 -- not less than 6 months. American stems are stable to 0.5 percent phenol. The following is known about the fatherland stems Moscow #2 and #4: when heated to 50 degrees Centigrade, the virus perishes within an hour, to 60 degrees -- within 30 minutes, to 75 degrees -- within 10 minutes; a 5 percent solution of calcium hypochlorite, a 5 percent solution of phenol, a one percent solution of alum, each one of these inactivates the virus in 10 minutes. The same effect upon the virus will result from a 0.5 percent solution of sulfuric acid, a 0.2 percent solution of formalin, and a one percent solution of creolin.

Differentiation of the virus. Comparison of the various characteristics of the virus of horse encephalomyelitis with the

- 238 -
RESTRICTED

RESTRICTED

characteristics of other neurotropic viruses revealed its sharp difference from poliomyelitis, lymphocytic choriomeningitis, transmissible encephalitis. The unmistakable differences between the various stems of American encephalomyelitis (the eastern, the western, and Venezuela) and the virus of horse encephalomyelitis known in Europe under the name of the Horn disease, and also the USSR virus of horse encephalomyelitis, were also revealed.

The varieties of the American virus practically do not differ from each other by their pathogenic qualities, size, and cultivability on a chicken embryo, but reveal differences in serological reactions and in crossover immunity tests.

The neutralization test gives a positive result only with homologous sera. The complement fixation test with the antigen from the brain of a guinea pig is also positive when homologous sera are used. When the antigen from the brain of a mouse is used, a weak crossover reaction between the western and eastern varieties of the virus is manifested. The European virus stems, by their serological characteristics, are entirely different from the American varieties, but similar among themselves, and they reveal a bond with the virus of rabies, which fact was established for the first time by Soviet scientists in 1934 (Vyshelesskiy and Moskov). The Leningrad stem of the virus, which was studied in our laboratory, gives crossover immunity reactions with the viruses of the demyelination encephalitis and rabies, but these reactions are less pronounced than the reactions with the homologous virus.

Epidemiology. Cases of humans stricken with the disease were

- 232
RESTRICTED

RESTRICTED

recorded for the first time in 1732 in America. Three men, who were in close contact with stricken horses, were themselves stricken with this form of encephalitis. Subsequently, other individual cases of humans stricken with horse encephalomyelitis were described during the epizootic outbreaks of 1934, 1937, and 1938. The most extensive epidemic of horse encephalomyelitis among humans, involving 509 people, occurred in Manitoba in 1941, with the emphasis on rural areas.

The epidemics are strictly seasonal and appear in the course of the summer and early fall. The diffusion of endemic centers, the simultaneous appearance of the disease in places that are far apart, led to the assumption of the presence of a carrier. The paths for the propagation of the American horse encephalomyelitis were studied in sufficient detail. In 1941, Hammon and his collaborators precipitated the virus from mosquitoes *Culex tarsalis*, caught in the endemic areas, establishing thereby the fact that these mosquitoes are the natural virus carriers. However, the propagation of the disease is so widespread that the possibility of other species of mosquitoes participating in the carrying of the virus is not excluded. In recent years new possible carriers came into view. Under experimental conditions, the disease was carried from stricken to healthy animals through the bite of the ticks *Dermacentor andersoni* and *Dermacentor variabilis*.

The possibility of the disease being transmitted by mosquitoes was first indicated in the USSR in 1935 by Muratova. The subject was then studied by Sergiyev, Shubladze, Tiburskaya. In 1942, they proved by experiment that the virus can survive for long periods

RESTRICTED

RESTRICTED

in the organism of the *Culex pipiens* mosquito, and then transmitted, via the mosquito sting, to healthy animals. These experiments proved the potential possibility of the virus being carried by mosquitoes, with the implication that the mosquito species subjected to the tests were not the only carrier of the virus.

The participation of ticks in the transmission of the disease is also assumed. In 1943, the possibility of the infection being transmitted by the ticks *Dermacentor silvarum* was experimentally proved. In 1944, Petrishev and Levkovich precipitated a virus stems of horse encephalomyelitis from the ticks *Hyalomma anatolicum*, *Ornithodoros lahorensis*, *O. papillipes*. In 1945, Ishukov and Ishukova pointed out that the meadow tick *Dermacentor marginatus* can be successfully contaminated in the laboratory and then transmit the virus to healthy animals via its bite.

Immunity. Convalescence is accompanied by the accumulation of specific antibodies in the blood. These antibodies appear at a very early stage, and can be discovered by the complement fixation and neutralization tests on the 3rd or 4th day of the infection. They are discovered less regularly in the body liquids. In studying immunity under experimental conditions (American encephalomyelitis), the dependence between the resistance of the immunized animals to intracerebral contamination and the presence of antibodies in the central nervous system was established. However, in the cerebral tissues of the animals, antibodies were discovered only in the cases where their titration standard in the blood was high. A mathematical correlationship between the titration standards of the antibodies in the blood, in the cerebrospinal fluid, and in the

RESTRICTED

RESTRICTED

cerebral tissue (300:1:2.5) was made apparent.

The antibodies may be detected in the conventional manner by the neutralization and complement fixation tests. American authors indicate that, in the case of the American virus of horse encephalomyelitis, the most sensitive method of detecting the antibodies by the neutralization test, is the intraperitoneal inoculation with the mixture. This does not depend on the lower sensitivity of mice to the virus when inoculated by the above method, since the titration standard of the virus, when inoculated intracerebrally, is only 10 times higher than in intraperitoneal inoculation, while the neutralizing capacity of the serum in the latter type of inoculation is 1000 - 10,000 times greater.

Stable immunity is attained by inoculation not only with the active, but also with the formalized virus, which fact makes possible the use of various vaccines for prophylactic purposes.

Prophylaxis and therapy. The basic safety measures in epidemic areas are measures designed to protect the people from mosquitoes, such as window screening and fumigation of premises. According to some published data, good results are obtained by vaccination which is a practically applicable method of specific prophylaxis in the case of horses.

Laboratory diagnostics consists of the discovery of the antibodies and the precipitation of the virus with its subsequent identification. For the purposes of serological diagnostics, in addition to the serum, it is possible to utilize the spinocerebral fluid, even though the antibodies in the latter are found with less regularity. Since the antibodies begin to accumulate at an early

RESTRICTED

RESTRICTED

stage, blood may be taken for the neutralization test, as well as for the complement fixation test as early as the end of the first week from the beginning of the infection.

The Virus of Lymphocytic Choriomeningitis (Armstrong Disease).

General data. Lymphocytic choriomeningitis is an acute infectious disease of human which is almost invariably characterized by a favorable prognosis. The propagation of the infection is tied in with mice and, possibly, other animals as the natural virus carriers.

After an incubation period of 8 - 13 days, the sickness is manifested by various symptoms, with the complexity of meningeal symptoms as the most typical. For this particular reason the disease is also known under the name of acute aseptic meningitis. Meningeal phenomena of various degrees of intensity are accompanied by an accumulation of a great quantity of cellular elements in the cerebrospinal fluid. The body liquor of patients is transparent, bacteriologically sterile, flows out under accelerated pressure, and may contain up to 3000 cells in one cubic centimeter, the cells being mostly lymphocytes. The sickness runs its course in 5 - 10 days and only on rare occasions lasts 4 - 5 weeks. The meningo-encephalitic type is described in cases when the meningeal symptoms are complicated by a state of somnolence, a deep distortion of the reflexes, and very rarely by paralyses of the facial nerve. It seems that the sickness most frequently encountered is caused by the same inciter but without any symptoms on the part of the nervous system, the so-called "grippal" type of choriomeningitis.

- 243 -

RESTRICTED

RESTRICTED

The existence of this form of meningitis was established by the precipitation of such an inciter from the blood of patients manifesting symptoms of the gripe. Another confirmation is also the presence of chorionmeningitis antibodies in some people who never before registered any related disorders.

Individual letal cases of human chorionmeningitis with pathological transmutations, similar to those observed in the experimental contamination of monkeys, were described. Lesions of cerebral membranes, lymphocytic infiltration of vascular plexuses, perivascular infiltrations, and nuclear hemorrhagic are described. The latter were observed under the dura mater, in the lungs, in the kidneys, and in the walls of the intestines. In the lungs of experimentally contaminated monkeys there is frequently observed pneumonia, the presence of serous exudate and perivascular lymphocytic infiltration.

Precipitation of the virus. Armstrong and Lille precipitated the inciter of the disease for the first time in 1934 and proved its virus origin. This virus was discovered in the process of contaminating monkeys with the brain suspension of a human who died of encephalitis St. Louis. Rivers and Scott, in 1935, precipitated a virus of the same type from cerebrospinal fluid of 2 patients showing symptoms of meningitis. Subsequently, several more virus stems of lymphocytic chorionmeningitis analogous with the first ones were precipitated.

Pathogenic characteristics. Under experimental conditions, monkeys, guinea pigs, white mice, gray mice, and dogs are susceptible

- 244 -

RESTRICTED

RESTRICTED

to this virus, particularly when inoculated intracerebrally. In the case of monkeys, guinea pigs, and white mice it is possible to induce the infection also by intranasal, intraabdominal and subcutaneous inoculations. Additionally, guinea pigs can be contaminated by rubbing the virus into the undamaged skin. The virus of choriomeningitis is only relatively neurotropic. During the first days of the sickness, it is invariably in the blood and is detected in organs and tissues outside the nervous system. The sickness induced by the inoculation of the virus may run its course without involving the central nervous system. In experimentally contaminated animals the virus penetrates not only into the blood, the brain, and the spinal cord, but also into the salivary glands, the muscles, the lungs, the liver, the spleen, the kidneys, the suprarenal capsules, the bone marrow, the lymphatic glands, and the cerebrospinal fluid. Obviously the virus is disseminated with the bloodstream throughout the organism, inducing in many organs pathological transmutations in the form of interstitial and perivascular infiltrations and diffused nuclear hemorrhages. (Figure 4b which is a microphoto)

Figure 4b. Experimental lymphocytic choriomeningitis in a guinea pig. Inflammatory reaction of a vascular plexus. Stained in accordance with Unna (collection of the Institute of Virology)

In contaminated mice the virus is present in the blood, ~~in the blood and the urine. It was also discovered that a guinea~~
 pig can be infected with choriomeningitis by merely rubbing the

RESTRICTED

RESTRICTED

virus into the undamaged skin.

Filterability, size. The size of the virus of lymphocytic choriomeningitis, as determined by the method of ultrafiltration, is 40 - 60 millimicrons.

Cultivation. The virus can be cultivated in vitro in a medium containing the tissue of a developing chicken embryo. Successful cultivation is attained by contaminating the chorio-allantoic membrane of 11 - 12-day old chicken embryos. There are no characteristic macroscopic changes on the contaminated allantois, and only on rare occasions individual disseminated non-transparent spots are visible.

Stability. This virus is not stable to high temperature, but endures under low temperature conditions. Its long survival is attained by maintaining it in a 50 percent glycerol solution, or in a frozen state after it was vacuum-dried.

Differentiation of the virus. The virus of lymphocytic choriomeningitis is easily distinguished from all other neurotropic viruses, which attack humans, and also from the virus of spontaneous encephalomyelitis in mice, by its pathogenic characteristics. The virus of spontaneous encephalomyelitis is pathogenic only to mice while the virus of lymphocytic choriomeningitis is pathogenic to guinea pigs and monkeys.

Epidemiology. It is possible that the actual number of cases of lymphocytic choriomeningitis is greater than the official statistics show. In the case of many humans manifesting mild

- 246 - RESTRICTED

RESTRICTED

neurological symptoms of the sickness virus neutralizing antibodies were discovered.

Choriomeningitis is well known not only in the United States, but also in England, France, Japan. In Tunisia choriomeningitis was observed in white mice and it was accidentally carried over to a human during the prophylactic vaccination procedure against yellow fever.

As yet it has not been ascertained as to who plays the part of primary host to the infecter, man or mouse. For a long time the direct link between the contamination of humans and the disease in mice could not be established. Mass infections were established only among white mice.

In 1937 it was established that domestic mice, trapped in houses where there were choriomeningitis patients, were carriers of the virus (Armstrong and Smith).

It was established experimentally that in mice recovered from the disease, the virus is still disseminated with urine elimination and nasal secretions for many more months. It seems that the sickness in mice is of great significance in the epidemiology of choriomeningitis.

Lymphocytic choriomeningitis in humans is seasonal since it occurs mainly during the winter and early spring. As a rule the sickness ends in recovery. The prevailing age of patients is from 20 to 40. Some cases where the sickness struck at young children are on record.

- 247 -

RESTRICTED

RESTRICTED

Immunity. Recovery from the disease in humans and susceptible animals leaves in its wake a stable immunity which, in the case of humans, guinea pigs, and monkeys, runs parallel with the formation of antibodies in the serum, while in the serum of immune mice no antibodies are detected.

The Virus of Acute Human Encephalomyelitis

General data. Acute disseminated encephalomyelitis is a grave disease which strikes most frequently at young people, runs a heavy course accompanied by a disseminated infection of cells and communication paths of the central nervous system.

The sickness is preceded by prodromal symptoms: headache, paresthesia in various parts of the body, general indisposition, weakness in the extremities, temperature rise. These general infection and brain symptoms are later on joined by symptoms of local affections in the nervous system: motorial disturbances, disruption of the function of the optical nerve, diminution of the auditory capacity. Some cytosis, with lymphocytes predominating, takes place in the cerebrospinal fluid. An increase in pressure occurs rarely. Mortality ranges from 10 to 32 percent. Generally speaking, no residual phenomena are observed in cases of recovery. In rare cases, however, the following after-effects remain: hemiparesis, defective speech, and diminution of intellect.

Pathomorphological examinations of the brain of human victims of the disease showed diffused inflammatory phenomena in the brain, spinal cord, and the peripheral sections of the nervous system. The basic and permanent indications are micronecrosis and demyelination.

RESTRICTED

RESTRICTED

an independent microbiotic process which is not linked to the vasoinflammatory process and can also be evolved in the absence of the latter.

Precipitation of the virus. The virus of acute encephalomyelitis was first precipitated in 1942 by Nareulis, Solov'yev, and Shablazov from the blood of a patient in the acute stage of the disease. The virus was precipitated in 1943, for the second time, from the brain of a woman who succumbed while showing symptoms of acute encephalomyelitis. For the third time, the virus was precipitated in 1945 also from the brain of a woman who succumbed while in the critical stage of the disease. These 3 virus strains turned out to be identical and were designated as SV, NF, and 70 strains. The laboratory study of the various characteristics of these strains led to their identification as the initiators of acute disseminated human encephalomyelitis.

Pathogenic characteristics. White and gray mice, rats, guinea pigs, rabbits, puppies, and monkeys. Contamination can be effected by intracerebral, subcutaneous, intraperitoneal, and intravenous inoculations, it being the case that in large animals (monkeys) even heavy dosages of virus, inoculated subcutaneously, will not necessarily induce the disease. Birds, such as young chicks, goldfinches, greenfinches, yellow buntings, chaffinches, reopolls, become infected through intracerebral inoculation, but not always, and this only after a long incubation period. Pigeons, sparrows, mountain finches, and hens turned out to be non-susceptible. The susceptibility of animals to this virus by age is characteristic. While it is impossible to induce the disease in adult dogs and hens, chicks and

RESTRICTED

RESTRICTED

puppies are susceptible to the virus.

By clinical symptoms, the disease is monotypic in all animals. On the 4 - 5th day, mice become sluggish, their wool becomes disheveled, tremor appears. At a later stage the involuntary movement of the anterior and posterior paws sets in, followed by clonic convulsions and death. In larger animals, the first indication of the sickness is a wobbly gait, the following symptoms being similar to the above described symptoms in mice. Paralyzed develop rather infrequently.

Regardless of the method of inoculation, the highest concentration of the virus is found in the brain. The presence of the virus in the blood and in the internal organs is not continuous even during the height of the malady.

The pathomorphological setup in the brain of contaminated animals, particularly monkeys, dogs, and rabbits, corresponds to the above described transmutations in the case of humans. (See figures 42 and 50 showing the processes of demyelination and micro necrosis, respectively.) In the brain of guinea pigs, rats, and mice, only inflammatory phenomena and micro necroses are manifested, while demyelination does not occur.

Filterability. The virus passes freely through Zeyts filters, Berkefeld V, N candles, and Chamberland L₃ and L₁₃.

Cultivation. The cultivation of the virus is successful in an 8 - 10-day old chicken embryo by contaminating it into the yolk sac or into the allantoic membrane, with the titration standards of the virus

- 280 -

RESTRICTED

NOTED

not attaining high concentrations. The embryo in the contaminated egg survives but some of the hatched chicks develop a clinically pronounced case of the malady.

Stability. The virus can be well preserved in a 50 percent glycerin solution. Vacuum-dried, it remains active for no less than 3 years. In a 10 percent cerebral suspension taken from stricken animals, the virus of human encephalomyelitis remains viable for 15 days at 37 degrees Centigrade, for 30 days at 20 degrees, for 60 days at 4 degrees.

A particular characteristic of this virus is considerable stability to temperature. It is able to withstand a water bath at 40 degrees Centigrade for 1 1/2 hours, at 75 degrees for 45 minutes. Boiling inactivates the virus in 2 - 5 minutes. Ultraviolet irradiation is lethal to the virus and disintegrates it within 10 minutes.

A 2 percent lysol solution fully inactivates the virus in 5 minutes, a 3 percent solution -- in 3 minutes, a 2 percent phenol solution -- in 30 minutes, a 3 percent phenol solution -- in 3 minutes. In alcohol the virus will perish in 30 minutes, in acetone and ether -- within an hour, in a one percent soap emulsion -- within 10 minutes.

Under the photodynamic effect of methylene blue, the virus becomes inactivated within 15 minutes.

Differentiation of the virus. The virus of acute human encephalomyelitis, by its characteristics, is sharply different from the viruses of the transmissible encephalitises, herpato-encephalitis,

- 267 -

RESTRICTED

RESTRICTED

and lymphocytic choriomeningitis. In neutralization and complement fixation tests, antigenic links have been established between the viruses of acute human encephalomyelitis, rabies, silver-black fox encephalitis, and horse encephalomyelitis. These links are more pronounced in crossover serological reactions with sera from immunized animals, and less pronounced when sera from convalescents are used.

Because of the identity in the pathomorphological setup between acute human encephalomyelitis and multiple sclerosis, the leading indication of either one being the presence of multiple nuclear demyelination, and also the proximity of the entire clinical syndrome for the two maladies, the problem of differentiation is important. The discovery of the specific virus of acute human encephalomyelitis made it possible to study the relationship between the above two maladies experimentally. It turned out that the blood serum taken from a considerable number of people stricken with multiple sclerosis possesses neutralizing properties with relation to the virus of acute encephalomyelitis. Such antibodies are discovered in at least one half of all multiple sclerosis patients examined. Thus, the similarity of clinical symptoms, the identity of the pathomorphological setup, and the experimentally derived serological data, made it possible for Margulis, Solov'yev, and Shubladze to establish the close relationship between acute human encephalomyelitis and multiple sclerosis.

Epidemiology. The number of cases of acute encephalomyelitis on record amounts to 8 - 9 percent of all neuroinfections. Most of the cases occur during the winter and in early spring. The favored

- 252 -

RESTRICTED

RESTRICTED

age is 20 - 30. The malady occurs sporadically but small epidemics as described in various countries are on record. As yet there is no clear concept as to how the infection is propagated.

Immunity. In 75 percent of all cases of recovery, antibodies are discovered in the serum. Recurrence is difficult to judge, since the sickness is frequently aggravated. In the experimental study of immunity in animals, it was established that the antibodies are one of the indicators of immunity. Animals with a high antibodies titration standard in their blood are non-susceptible even to intracerebral inoculation of the virus.

Therapy. Marcellis, Solov'yev, and Shublausk suggested that a vaccine prepared from the cerebral tissue of contaminated animals be used as a therapeutic agent.

II. DEMOTROPIC VIRUSES

The segregation of this group of viruses is more conformant with the unification principle by the index of tropism. The "demotropic viruses", although usually striking at the skin and the mucous membranes, may also affect other organs and tissues. The virus of herpes, when carried onto the cornea or when inoculated into animals intracerebrally, induces the affection of the central nervous system and the development of encephalitis resulting in death. The virus of smallpox vaccine can be adapted to the brain of a rabbit and will induce encephalitis with the complete absence of any dermal lesions. Nevertheless, under natural conditions, when striking at humans, these viruses will, as a rule, induce various

RESTRICTED

lesions of the skin and mucous membranes.

In addition to the known and well studied viruses, such as the initiators of smallpox and herpes, the virus origin of the following diseases has been established: chicken pox, shingles, blepharitis, keratoconjunctivitis, and trachoma. We leave out the individual description of these, since the characteristics of their initiators have been inadequately studied, and their virus origin proved mainly by morphological methods.

The virus of chicken pox was discovered in the form of elementary corpuscles in the discharge of the vesicular eruption of the patients. When stained by the use of the Papanicolaou method, these corpuscles become visible as formations of exceedingly small cocci, disposed in pairs, or forming short chains. The histological manifestation of the malady is characterized by the presence of intranuclear acidophilous inclusions in the epithelial cells. Attempts to transmit the disease to animals were not successful, with the exception, as reported by others, of the discovery of intracellular inclusions in the testicles of monkeys upon being inoculated with a substance taken from patients. The complement fixation test is also successful. Chicken pox is an acute infectious disease which strikes through the mucous membranes of the respiratory paths, selecting mostly children up to the age of 15.

The virus of shingles (zona or herpes zoster), in the form of elementary corpuscles, similar to the virus of chicken pox, becomes visible through the use of special methods of staining the material taken from the vesicular lesions during the first 48 hours

of the sickness. Morphologically these viruses are indistinguishable from one another. There is also no reliable data on the reproduction of these infections on animals. It is only necessary to take note of the experiment byloodpasteur, who successfully cultivated the virus on a piece of human skin transplanted onto the chorioallantois of a developing chicken embryo. In addition to the morphological similarity between chicken pox and herpes zoster (elementary corpuscles, inclusions), there is also immunological proximity between these two infections. After recovery from chicken pox, children are immune to herpes zoster, and after recovery from herpes zoster, children are immune to chicken pox. There are also essential differences: chicken pox is highly contagious, while herpes zoster is not. It seems that while the viruses of chicken pox and herpes zoster are akin to one another they are not identical.

The virus of blennorrhoea in the newborn forms typical intraplasmatic inclusions in the cells of the conjunctival epithelium. The elementary corpuscles contained in the inclusions are formations having a diameter of 0.25 microns.

This malady is encountered in the newborn and sometimes in adults and is characterized by an acute first stage followed by a course of long duration -- from 3 to 12 months. Newborn are usually attacked by it 5 - 9 days after birth. The disease can be transmitted to monkeys.

The virus of keratoconjunctivitis is found in the discharge from the conjunctiva of patients, can be preserved in tissue cultures, and can be adapted to the brain of mice. After 2 - 7 days of the

RESTRICTED

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intracerebral inoculation or else there is the setting in of convulsions followed by paralysis and death within 24 hours from the appearance of the first symptoms. It is assumed that the diameter of the virus is about 25 millimicrons. It can be neutralized by a serum prepared from convalescents. This disease occurs rarely, is characterized by the reddening and edema of the conjunctiva, more frequently of one eye and sometimes of both eyes. In addition to the inflammation of the conjunctiva, the patients suffer with headaches and general indisposition. The malady lasts 3 - 4 weeks, ending in recovery. Complications in the form of scar formations on the cornea are rare.

The virus etiology of trachoma has been adequately proven. There is available data on finding the trachoma virus in the form of elementary corpuscles of a 0.25 micron diameter disposed in the epithelial cells of the conjunctiva of patients. Intracellular inclusions are described. The characteristics of the virus are as follows: mild infectiousness even for humans, irregular filterability, high affinity to the tissue of the human conjunctiva, mild antigenic qualities. There are a series of reports on the contamination of monkeys with trachoma but there are doubts as to the reliability of such diagnosis since monkeys are known to be subject to spontaneous eye sicknesses which are similar to human trachoma.

The Virus of Smallpox (Variola Vera)

General data. Smallpox is a ~~grave and extremely infectious~~ disease for which, in addition to grave toxicoinfectious phenomena, a gangliovesicular eruption on the skin and on the mucous membranes

RESTRICTED

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is very characteristic.

The general characteristics of the onslaught of the disease are various. By the degree of gravity three varieties of smallpox are known: the medium variety, or variola vera; the heavy variety, or black smallpox (variola nigra); and the pseudo-smallpox, or varioloid.

The incubation period usually runs for 13 days, sometimes less. The malady begins with a prodromal period, which runs into 3 - 4 days, and is marked by fever, headaches, general weakness, frequent vomiting, and pains in the area of the sacrum. On the second and third day, as a forerunner, a skin rash in the form of red spots, or roseolae, the size of lentils or kernels, appears, first on the face, then on the body and extremities.

The prodromal rash may be of a different variety, such as petechiae, becoming localized in the area of the lower part of the abdomen and on the inner surface of the thighs. The counterdevelopment of this rash is effected in 2 - 3 days. By the end of the third day the gangliovesicular eruption, so characteristic of smallpox, appears. It is most closely disseminated on the face and on the extremities. After 2 - 3 days the ganglia are transformed into vesicles filled with a transparent fluid. Beginning with the ninth day the fluid in the vesicles becomes turbid due to the admixture of leucocytes and is transformed into pus. This is the gravest phase of the infection. In the course of this phase some patients die from complications, such as bronchitis, edema of the larynx, or septicopyemia, which develops as a result of a secondary bacterial

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RESTRICTED

infection. The pustules begin to dry on the 11th or 12th day and this is accompanied by exasperating itching. The falling away of the scabs, formed during the drying of the pustules, takes place beginning with the 30th - 40th day, leaving drawn in scars in their place. Very typical of smallpox is the peculiar fever curve of the patient. To the moment of appearance of the poxmark rash, the temperature drops to 31 degrees Centigrade and below, during the pus formation period it rises again and then, step by step, it drops back to normal as the pustules become dry. The duration of the disease in non-complicated cases is 5 - 6 weeks.

Complications which may occur during natural smallpox are numerous and varied and may lead to grave consequences. Persons recovered from this dread disease frequently remain with mutilating scars on their skin and are sometimes afflicted with blindness and deafness for the rest of their lives. The mortality rate is 25 - 30 percent.

- 258 -

RESTRICTED
RESTRICTED

RESTRICTED

Pathoanatomical examinations of internal organs show edemas of the mucous membranes of the respiratory system and the alimentary canal, and frequently hemorrhages. On the mucous membranes of the pharynx, bronchial tubes, and esophagus, specific smallpox lesions analogous to same on the skin, are discovered. In the lung tissues, in severe cases, extensive hemorrhages, atelectatic zones, and gangrene are observed. In the liver there is fat regeneration, the spleen usually does not undergo any changes. In some cases septic infarcts are described. The muscle of the heart shows edema, and the pericardium shows petechial hemorrhages.

The precipitation of the virus. In 1892, Guarneri described microscopic corpuscles, which subsequently were called by his name, disposed in the protoplasm of the epithelial cells of the smallpox lesions. The corpuscles can be stained well with hematoxylin-eosin. Guarneri relegated them to the protozoa and assumed that they propagate by cell division and sporogenesis. This theory prevailed until such time as Pashen, in studying the Guarneri corpuscles, came to the conclusion that the small formations inside the Guarneri corpuscles, which were considered heretofore to be spores, are in reality the elementary corpuscles of the smallpox virus. Subsequently, additional data proving the etiological part played by the above elementary corpuscles (the Pashen corpuscles) were obtained. By differential centrifuging it is possible to obtain a pure (free from tissue elements) suspension of these elementary corpuscles. An experimental contamination was successfully effected with the aid of one

RESTRICTED

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elementary corpuscle, which fact finally confirmed the identity of the elementary corpuscles with the virus.

The precipitation of the elementary bodies in their pure state made it possible to study their chemical structure. It turned out that the elementary corpuscles consisted of albumina, fat, hydrocarbons, some copper, riboflavin, and biotin.

Pathogenic characteristics. The reproduction of a malady, similar to human smallpox, was successful only in individual cases, on monkeys. The inoculation of conventional laboratory animals, such as rabbits, guinea pigs, with the virus of smallpox results only in local lesions.

When monkeys are contaminated onto a scarified skin or intradermally, they manifest on the 3-5th day specific skin lesions, which are analogous with some in the case of humans. The contamination onto the cornea of the eye produces keratitis. An intravenous contamination may produce a generalized rash on the 5th day. In addition, monkeys can be contaminated via the scarified mucous membrane of the nose, lips, and by inhaling the infectious material.

The contamination of rabbits directly from a human can be accomplished regularly by placing the infectious material upon the cornea, and less regularly by intradermal contamination. In subsequent passages, the virus takes easily when inoculated subcutaneously. On the third day after the smallpox material has been carried onto the scarified skin, there occurs the formation

RESTRICTED

of papules, which, by the 5th day, are transformed into vesicles, and then into pustules (See Figure 51). On the 8-10th day the dried pus scabs begin to fall off. The same process occurs in the case of guinea pigs and calves. In the protoplasm of the epithelial cells, at the places of lesions, the characteristic inclusions and elementary corpuscles are to be found. Through the inoculation of the smallpox virus into animals, such as rabbits, calves, monkeys, and others, its qualitative characteristics undergo a change. The virus loses its ability to induce a generalized infection in humans, and becomes capable of producing only local lesions. Such a modified virus is known as the virus of the vaccine. This term is without precision, since, literally speaking, vaccine means cowpox, a virus disease, stipulated by an inciter that is very close, if not even identical, with the virus of human smallpox, yet inducing in humans a local process only. By analogy, the virus of vaccine began to be designated as the virus of smallpox, which, in the indicated sense, has changed its characteristics after having been passaged through the organism of animals.

Filterability, size, morphology. The virus of smallpox belongs in the category of large viruses. Its size, as determined by the method of ultrafiltration through diaphragms, equals 125-175 millimicrons, and, as determined by the method of ultracentrifuging, it equals 170-180 millimicrons. The elementary corpuscles can be observed with the aid of a conventional microscope, when adequately effective staining methods are employed

staining-silvering as per Horozov, Pashen, Romanovskiy-Gienska). The most precise concepts on the sizes and the form of the elementary corpuscles have been now obtained with the aid of the electronic microscope.

The elementary corpuscles are rectangular in shape, restricted by an outer membrane, and of uniform size. In each elementary corpuscle there are five darker zones. After treating the elementary corpuscles with a caustic alkaline solution, which partially dissolves the nucleoprotein, their shape becomes round, the outer membrane becomes less compact, and the dark spots become more pronounced, resembling polynuclear leucocytes.

Horozov proved the existence of two biological varieties of the virus of vaccine differing also morphologically (large-cellular and small-cellular), with the first one having a higher virulence. In the initial state of the basic process the large-cellular variety predominates.

The smallpox virus induces the formation of intracellular inclusions, which were designated, by the name of the man who described them, as the Guarnieri corpuscles. The Guarnieri corpuscles are disposed in the protoplasm and are sharply visible due to the presence of a light rimlet. Their shape varies: round, fusiform, oval. Inside the Guarnieri corpuscles, the elementary corpuscles (Pashen corpuscles) are frequently found. In accordance with present concepts, the Guarnieri corpuscles are, most probably, virus colonies. These inclusions are strictly

specific for smallpox, and their detection has indubitable value for diagnostic purposes.

Cultivation. Cultures of the virus of vaccine in surviving tissues were obtained by the Mayland method. It can also be cultivated successfully on a developing chicken embryo. In this process, specific transmutations in the form of whitish opaque foci, with crater-like depressions in the center, appear on the chorioallantois. Under the microscope, elementary corpuscles and Guarnieri corpuscles can be seen in the cells of the tissue cultures and on the chorioallantois of the embryo.

Stability. The virus of smallpox is very stable with relation to external influences. Of particular interest is its exceptional stability to desiccation, which fact has an important epidemiological significance. In a desiccated state, the stability of the virus is increased. In the form of a suspension in a physiological solution, at 60 degrees Centigrade, the virus becomes disintegrated in 30 minutes, but in the form of the desiccated smallpox pustule scabs it can survive heating up to 100 degrees Centigrade for 5-10 minutes. The virus-containing vesicular fluid, when kept at room temperature and in the dark, retains its infectious characteristics for 3 months. Smallpox pustule scabs, under the same conditions, remain infectious for over a year.

Like other viruses, the smallpox virus survives well under low temperatures.

- 263 - RESTRICTED

RESTRICTED

The virus is comparatively stable to phenol: a 5 percent solution of the latter will not disintegrate it in an hour. Hydrochloric acid 1:1000, caustic soda (N/500) will inactivate the virus within an hour; alcohol, ether, acetone--within half an hour. Mercuric chloride 1:5000 and potassium permanganate will disintegrate the virus within 10 minutes.

Differentiation. In addition to human smallpox, there are many varieties of smallpox in animals and birds: vaccinia, or cowpox, "vaccinia", or ass-pox, "ovinia", or sheep-pox, equinia, or horse pox, "lapinia", or rabbit pox, arinia, or bird pox. The clinical manifestations of smallpox in animals are different from those of human smallpox, although the viruses are similar, particularly by their antigenic and morphological characteristics. For purposes of differential diagnostics, the animal forms of smallpox have no practical significance, since no contamination of humans by natural animal smallpox is on record.

The mutual relationship between the viruses of humans and animals was studied in more detail in the case of the virus of vaccinia. From the days of Jenner the virus of cowpox has been in use for the immunization of humans. Among the numerous stems of vaccinia, there probably are, in addition to the virus of spontaneous cowpox, also modified varieties of the virus of human smallpox that passed through the organism of a calf. There is even a theory that, in general, there is no such thing as spontaneous cowpox, and that the cow disease stems from humans. This is a difficult problem to solve, since by size, morphology,

RESTRICTED

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and serological reactions, the two viruses involved are indistinguishable. There is some data to the effect that, in the case of crossover depletion in elementary corpuscles on the part of the viruses of human smallpox, vaccinia, and spontaneous cowpox, it becomes possible to establish some sort of differences in the antigenic structure of the above viruses. These differences, however may be present simply within the limits of different varieties of the identical virus.

In all experimental work, the investigator usually deals not with the virus of natural smallpox but with its variety obtained through the passage of the smallpox virus through the organism of a calf, rabbit, monkey, or other animal. All these viruses, as already mentioned above, are called viruses of vaccinia, or, as suggested by Morozov, viruses of smallpox vaccine.

A disease very closely related to smallpox is alastrim, or milkpox. Clinically, alastrim differs from smallpox by its mild course and low mortality rate (0.5 percent). Elementary corpuscles resembling those of smallpox are found in the skin lesions. There are crossover serological and immunity reactions between smallpox and alastrim. There is one opinion to the effect that alastrim is merely a variety of smallpox (variola minor).

The virus of chicken pox is so close to the virus of smallpox that the differential clinical diagnosis is sometimes very difficult, particularly in the cases of the mild form of smallpox (varioid). Virusoscopy then comes to the aid of the clinical

RESTRICTED

RESTRICTED

diagnosis. In the pathological formations caused by chicken pox, as well as by natural smallpox, elementary corpuscles are found. But the elementary corpuscles of chicken pox are easily distinguishable from the elementary corpuscles of smallpox; they are polymorphous and are not disposed in the form of aggregates. They are smaller in size than the Papan corpuscles. Also, in chicken pox the Paul phenomenon (see below) is absent.

In some cases it is necessary to differentiate smallpox from herpes. The distinguishing feature is the presence of intranuclear inclusions in herpes and cytoplasmic inclusions in smallpox.

The staining of the material from the vesicles with Victoria blue, by the Gerzberg method, is used successfully for rapid microdiagnosis. The elementary corpuscles of smallpox, being larger, become stained more rapidly than the elementary corpuscles of herpes. It was established that the virus of smallpox becomes stained in 5-10 minutes, the virus of chicken pox--in 20 minutes, and the virus of herpes--in 30 minutes.

Epidemiology. Epidemics of smallpox are known to mankind since times immemorial. It seems that the most ancient epidemiological center was Central Africa. There is also a basis for believing that, 2000 years before our era, smallpox existed in India and China. In Russia, smallpox made its appearance in the XV - XVI centuries, and attained its maximum propagation in the XVIII century. Prior to the great October revolution, 100,000

RESTRICTED

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150,000 people were stricken with smallpox annually, with 40,000-50,000 being the average toll of the dead per year.

After the revolution, due to energetic practical counter-measures and the great progress of scientific research in this field, smallpox morbidity began to decline sharply, and already, beginning with 1920, not a single case of natural smallpox was registered in the USSR.

In 1919, V. I. Lenin signed the decree on compulsory vaccination. The second decree, elaborating upon the first one, was put into force in 1924. By a decree of the Council of Peoples' Commissars, USSR, 1929, the following fixed periods of vaccination and revaccination against smallpox were established: vaccination of children within one year from birth, revaccination at the ages of 4-5, 10-11, 16-20. In addition, the following countermeasures were instituted: the compulsory reporting to the authorities of all cases of smallpox, hospitalization of patients and suspects, compulsory microbiological diagnosis via laboratory studies of all sicknesses, which may appear suspect.

The source for the propagation of the infection is the stricken patient. The virus of smallpox is present in all affected organs. The virus spreads outward via the pus, nose and mouth mucus secretions, and partly via the urine and feces of patients. Of particularly great epidemiological significance is the dissemination of the viruses from the mucous membrane surfaces in conversation and coughing. The smallpox patient is a...

- 267 RESTRICTED

RESTRICTED

source of infection during the entire course of the malady, beginning with the incubation period and ending with the falling off of the scabs.

Contamination occurs through direct contact with patients, through third persons, and through articles that were used by patients. Through its high resistance to external influences, particularly desiccation, the virus of smallpox, on various articles, remains dangerous for a long time.

The gateways for the infection are the upper respiratory paths and damaged areas in the external covering of the body, with the greater epidemiological emphasis falling to the first.

The serology curves in foreign countries, which are endemic with relation to smallpox, show regular peaks every 5-6 years, stipulated by the natural increase in the population and as yet non-immunized interlayer of the latter.

Immunity. Smallpox usually leaves in its wake a lifelong stable immunity. There are, however, individual cases of recurrence after 20 years. Stable immunity can be created by vaccination with live smallpox vaccine, first introduced by Jenner. Vaccination immunity is less durable, becoming extinguished, on the average, in 7 years.

The presence of agglutinins, precipitins, neutralization and complement fixation antibodies in the blood of ~~reconvalescent~~ and vaccinated persons has been proved. The diversity of serological reactions with the virus of smallpox vaccine can be explained by the

RESTRICTED

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complex antigenic structure of the latter. To date, the presence of five antigens has been established.

The L S antigen is a water soluble albuminous complex, which consists of two antigens differing from each other by their thermal stability: L antigen is thermolabile, and becomes inactivated at 50 degrees Centigrade, S antigen is thermostable, and can withstand heating up to 90 degrees Centigrade. Antigens L and S take part in the reactions of precipitation, agglutination, and complement fixation. The nucleoprotein antigen NP is a precipitogen, the antigen X is an agglutinogen and an antigen that entered into the reaction of neutralization. The last two antigens were not obtained in their pure state, their chemical nature is not yet finally determined, and their presence can only be judged by serological reactions with sera, depleted by other enumerated antigens.

In the experimental study of the mechanism of immunity, induced by smallpox vaccine, observations of great importance and interest were made by Morozov. He proved the presence of a specific lysis of elementary corpuscles under the effect of hyperimmunized sera in vitro and in vivo. The following stages of lysis were established: (1) the swelling of the corpuscles, (2) the appearance on their inside of one or several fanlike shaped dark inclusions, (3) loss of capacity to become stained, (4) disintegration and disappearance of the corpuscles. In the study of the vaccinal process under the microscope, the gradual disintegration and disappearance of the Guarneri corpuscles from

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the cornea of a rabbit was noticed on the 7-8th day after contamination. Korozov advanced the opinion that the essence of the interaction between the antibodies and the viruses is in the phenomenon of lysis.

There is a close relationship between immunity and the presence of antibodies. Nevertheless, the absence of antibodies in the blood does not always bespeak susceptibility. With the passing of time from the moment of recovery from the disease, or from the moment of a preventive vaccination, the concentration of antibodies in the blood becomes decreased, since the intensity of their formation is decreased, but they are preserved in adequate numbers in the tissues of the organism.

In the opinion of some authors, as the basis of immunity lies the action of the humoral antibodies. In the opinion of others, it is the ability of the cells and tissues to destroy the virus, which ability is acquired by the cells and tissues after vaccination. There is a third conception, according to which immunity is closely tied in with outlasting the virus in the immune organism. However, the experimental data collected to prove this are in contradiction with epidemiological observations and, as such, cannot be accepted.

Prophylactics and therapy. Even in ancient times, in order to prevent the severe natural malady of smallpox, variolation, or artificial contamination by carrying the pus from smallpox patients onto the mucous membrane of the nose, or into scratches on the skin,

- 276 -

RESTRICTED

RESTRICTED

was practiced. The resulting sickness usually ran a mild benevolent course, leaving in its wake a stable immunity. However, there was no assured safety in variolation, since it frequently led to severe stages of the disease and death.

The milkiest of weapons in the struggle against smallpox became the discovery by Edward Jenner of the prophylactic characteristics of cowpox. The method of vaccination and the method of preparation of the vaccine has undergone considerable changes from the days of Jenner. The smallpox vaccine, or smallpox detritus, is a scraping from the skin lesions of a contaminated animal, the scraping being mixed with glycerin and triturated into a homogeneous mass. Soviet scientists developed a series of methods for improving the effectiveness of vaccines. In the place of glycerin, as the conserving agent, Morozov suggested the use of chicken albumin, which rendered the preparation more thermostable. A particularly important accomplishment was the obtaining of a dry smallpox vaccine of exceptional thermal stability and durable freshness, for a period of 1-1½ years, instead of 4-6 months for the conventional glycerin vaccine. Post-vaccination immunity persists for 7 years, then disappears, calling for revaccinations in order to sustain continuous non-susceptibility.

RESTRICTED

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Laboratory diagnostics. The laboratory diagnosis of smallpox is based on the morphological discovery of the Pashen corpuscles in the pathological material, or on the biological test on animals (the Paul method).

The material for investigation comes from the discharges of the mucous membranes of the nose and pharynx, the contents of the papules, vesicles and pustules, scales and scabs. The examination should begin with the preparation of smears, silver-staining of the latter by the Morozov method, in order to discover the presence of the Pashen corpuscles. In case of a negative or doubtful result of the microscopic examination, the Paul biological test is to be resorted to. This test is conducted as follows. One drop of the material being tested is placed on the scarified cornea of a rabbit. If the virus is present in the material, there will, after 36 - 48 hours, occur the formation, along the line of the scar, of specific smallpox lesions: small, round, and transparent nodules. In another 36 - 48 hours, due to the desiccation of the epithelium, crater-like concavities appear in the centers of the nodules. These lesions become particularly pronounced when the eyeball, after being removed, is immersed into mercuric chloride alcohol (4 grams of mercuric chloride, 30 cubic centimeters of ethyl alcohol, 70 cubic centimeters of distilled water). (For details see special instructions of the Ministry of Health, USSR, which were compiled by M. A. Morozov.)

The Virus of Herpes

General data. Herpetic Fever, or simple herpes (herpes simplex), is a febrile disease, accompanied by a vesicular rash on the skin.

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membranes and skin, usually localized on the face near the lips and the nostrils, on the eyelids, and, more rarely, in other places.

The vesicles are filled with transparent or, less frequently, somewhat cloudy or blood-containing substance. After some time these dry forming incrustations that fall off without leaving any scars.

Fever attains at times 39 - 40 degrees Centigrade. Pains in the muscles and joints, also dyspepsia, are observed frequently.

Excepting headaches, there are no other manifestations on the part of the nervous system. The sickness continues for 1 - 2 days without leaving in its wake any notable consequences. Very frequently the herpetic rash accompanies other sicknesses, such as malaria fever, croupous pneumonia, anjina, grippe, meningitis. Histological examinations of herpetic vesicles show that the transformations consist of fibrinous inflammation of the epidermis, which becomes easily separable from the lower part of the edematous tissue. The vesicular fluid contains polymorphous nuclear cells, large mononuclear and epithelial cells, and very small basophilously stainable formations.

Herpetic inclusions were first described by Lishchynskiy in 1921 as observed in the nucleoli of the epithelial cells, located on the inner wall of the vesicles. They are eosinophilic grains of various dimensions.

Precipitation of the inciter. The virus of herpes was discovered for the first time in the fluid of a herpetic vesicle in a human in 1912 (Grüttner), by way of contaminating, with this fluid, a rabbit via the scarified cornea of the eye. The virus was passed from rabbit to rabbit, the passage invariably resulting in keratoconjunctivitis.

RESTRICTED

Pathogenic characteristics. The virus of herpes, when inoculated onto the scarified cornea or skin of rabbits and guinea pigs, causes the formation of characteristically herpetic vesicles, manifesting thereby its dermatropic character. It was, however, noted that, frequently, rabbits, which underwent contamination via the cornea, manifested some central nervous system symptoms, such as convulsion, nervous moving about in circles, excessive salivation, and gnashing of the teeth. The virus was precipitated from the brain of animals.

The neurotropic characteristics of the virus are also manifested when intracerebral contamination is used on guinea pigs, kittens, dogs, monkeys, rats, and mice. Whether the virus of herpes will cause encephalitis in humans is still an open question.

Experimental contamination of animals with the herpes virus via the skin established the dermatropic characteristics of the virus; contamination via the scarified cornea of the eye demonstrated its keratogenic properties; intracerebral inoculations proved its neurotropic characteristics with relation to numerous animals. Consequently herpes is not a localized disease in the rigid sense of the word. This conclusion is confirmed by the fact that the virus is also found in the blood of animals contaminated via the cornea and intracerebrally.

Pathomorphological transmutations in herpetic keratitis of rabbits are manifested via an inflammatory reaction of the cornea of the eye accompanied by polymorphous nuclear infiltration. The presence of intranuclear eosinophilous inclusions in the epithelial cells of the cornea is characteristic. These inclusions appear ?

RESTRICTED

hours after contamination and attain their numerical maximum within 24 hours.

Herpetic encephalitis in animals is accompanied by phenomena of meningitis, of inflammatory perivascular infiltrates, consisting of lymphocytic glia cells, the presence of lymphocytic infiltration of membranes, etc. In the neurons and the glia cells, and sometimes in the epithelial vascular cells, intranuclear corpuscles or inclusions are found.

Size, filterability, morphology. The virus passes through Berkefeld candles V, through Chamberlain L₁ and L₂. Its sizes, as determined by filtration through graticulation diaphragms, range from 100 to 150 millimicrons.

The morphology of the herpetic infection was studied in detail. The presence of cytoplasmic inclusions in the cellular nucleoli of the stricken organ is pathognomonic. By their etiological significance, these formations carry considerable specific weight in the diagnostics of the malady comparable to the importance of the Negri corpuscles in rabies.

Cultivation. The virus of herpes develops successfully on tissue cultures and on the chorioallantois of a chicken embryo. In chorioallantoic contamination, macroscopic transmutations of the membrane in 9 - 17-day old embryos can be observed. These transmutations are not encountered in younger and in older embryos. The transmutations are large nuclei of proliferative-necrotic structure with a central area of necrosis. Microscopically these are transmutations of sharply accentuated necrosis, proliferation of epithelium, and inflammatory phenomena in the mesoderm. The specific corpuscles

RESTRICTED

of the inclusions may also be discovered in the ectodermal cells.

The first passages of the virus are usually not lethal to the embryo. The subsequent ones kill the embryo by the 3rd - 5th day upon contamination. Some strains of the herpes virus, after more than 30 passages on the chorioallantois, are not pathogenic any longer to mice. The specific inclusions were discovered frequently in the heart, liver, spleen, kidneys, lungs, and in the vascular endothelium of chicken embryos. The cultivation of the virus of herpes via the contamination of the embryos into the amniotic chamber, into the yolk sac, and into the brain, is also effective.

Stability. In a desiccated state, the virus remains active for years, in 50 percent glycerin it remains active for several months. It is not very stable to temperature. When exposed to a temperature of 56 degrees Centigrade for 30 minutes, or a temperature of 37 degrees for 24 hours, it will disintegrate. The virus is also sensitive to oxidation.

Differentiation of the virus. The virus of herpes differs from other dermatropic viruses by clinical, pathomorphological, and antigenic characteristics. Some immunological links, however, were established with the virus of smallpox: the cornea of a rabbit, immunized by the virus of herpes, is partially non-susceptible to smallpox contamination, and in the blood serum of animals which are immune to herpes there are smallpox neutralizing antibodies.

By clinical manifestations there is some similarity between the virus of herpes and the virus of zona (herpes zoster). However, this

RESTRICTED

similarity is very insignificant. The vesicles, common in the case of zoster, are larger and they are frequently disposed along the run of the nerve branches, or along the nerve stem proper, it being the case that the appearance of these vesicles is accompanied by neuralgia. Their secondary appearance is very rare, although minute herpetic vesicles may appear frequently on the same human. The virus of herpes induces characteristic transmissions in animals. The virus of the zoster will not contaminate animals. Crossover immunological reactions are also absent.

Certain tests were reported to the effect that rabbits, contaminated with herpes via the cornea, developed encephalitis, and this furnished the reason for comparing the virus of herpes with the virus of epidemic encephalitis leucogenus. However, no reliable data on the etiological part played by the virus of herpes in epidemic encephalitis is available. Also, the theory on the virus origin of the latter disease has not yet been proved directly.

Epidemiology and immunity. No epidemic propagation of herpes was reported. The virus is widely disseminated among humans, and it can remain in the organism for a long time without inducing the disease.

In the opinion of some authors, the immunity in herpes is of the infectious variety, i.e., the specific presence of the virus stipulates the non-susceptibility to a new infection. This is confirmed by the fact that, after recovery, antibodies are present in low titration standard only. It is possible, through immunization, to induce the accumulation of a small number of antibodies in

experimental animals. The mixture of immune serum and virus is not pathogenic to animals, and, when inoculated into a developing chicken embryo, will not induce the formation of specific transclusions, bearing witness to the propagation of the virus. In addition to the virus neutralizing antibodies, there are also complement fixation antibodies.

Laboratory diagnostics. The laboratory material (the fluid from the herpetic vesicles) is inoculated onto the scarified cornea and onto the scarified skin of a rabbit. In addition, it is inoculated into the brain of 2 - 3 rabbits and 3 - 5 white mice (with subsequent 3 passages of their cerebral tissue). For detailed virological research, passages of the material through chorio-allantoic cultures are effected. In order to discover the specific inclusions in the case of experimentally contaminated animals, histological examinations of vesicles and stricken organs and tissues of the cornea of the eye, skin, the brain of rabbits and mice, and the chorioallantois of a chicken embryo, are conducted.

III. PNEUMOTROPIC VIRUSES

To the group of pneumotropic viruses belong the members of virus infecting striking predominantly at the respiratory organs. They are the viruses of epidemic grippe A and B, the virus of psittacosis, the virus of the common cold. At this point it is necessary to mention the so-called atypical pneumonias.

The etiology of atypical pneumonias is not yet clear. This malady most frequently strikes at young people. As differentiated

RESTRICTED

from the pneumonia of coccal origin, they are not susceptible to therapy with sulfamide preparations. After an incubation period of 2 - 3 weeks, the atypical pneumonia runs a mild or medium severity course, manifesting itself by fever, headache, and general indisposition. A steady symptom is a dry, at times paroxysmal cough which, toward the end of the sickness, is accompanied by the discharge of a muciform and purulent sputum, frequently containing small amounts of blood. An X-ray examination shows a darkening in the area at the root of the lung, which darkening is spreading fanwise to the periphery of the lung field. The average hospitalization period is 32 days, the prognosis is favorable, death occurs in rare cases only.

For purposes of diagnosis, the cold agglutination of erythrocytes test is used. A 2 percent suspension of human erythrocytes of Group I is mixed with the serum of the patient and allowed to stand at a temperature from 2 to 10 degrees Centigrade for 12 - 24 hours. When agglutination takes place, a positive result is indicated.

In searching for the inciter of atypical pneumonia, numerous investigations were conducted. However, the reports of the discovery of a filterable virus, as yet, did not receive the necessary approbation, since not in a single case was the clear mutual relationship between the precipitated agent and the given malady established. The virus etiology of atypical pneumonias is based on the possibility of the transmission of the infection to healthy people via the inhalation of filtered gargles from the respiratory paths of patients.

- 279 -

RESTRICTED

RESTRICTED

The Virus of Gripe

General data. Epidemic gripe is an acute infectious disease predominantly arising at the organs of respiration, particularly the upper respiratory paths. This malady occurs sporadically, but from time to time, at 2 - 4-year intervals, epidemic outbreaks occur.

The typical clinical manifestations of the non-complicated epidemic gripe are a sudden rise in temperature, rigor, headache, aching muscles, leucopenia. Catarrhal phenomena in the upper respiratory paths are most frequently expressed in a dry cough and a slight congestion of the mucous membrane. Basically, a state of general intoxication of the organism prevails. The sickness lasts from 3 to 7 days, followed by convalescence, unless there are complications of which the most dangerous are the pneumonias, which are stipulated both by the pathogenic effect of the virus as well as by secondary bacterial infection.

Pathologico-anatomical transmutations in pneumonia are varied. Abrikosov classifies them as follows: (a) diffuse serohemorrhagic pneumonia with inflammatory edema and hemorrhages; (b) multinuclear catarrhal bronchopneumonia; (c) draining catarrhal pneumonia; (d) fibroid and fibrinohemorrhagic pneumonia, with the discharge of fibrin into the alveoli and a general aspect of red hepatization; (e) desquamative pneumonia with the dissemination of the purulent process along the lymphatic vessels of the interlobular laminae.

Microscopical examinations disclose most frequently necroses of the bronchial and alveolar epithelium and of the vascular walls epithelium, with an accumulation of erythrocytes in the alveoli.

- 258 -
RESTRICTED

RESTRICTED

Precipitation of the virus. The virus of gripe was segregated for the first time by Smith, Andrews, and Luow (in 1933) with the aid of an intranasal inoculation with a pharynx gargle, taken from stricken patients, of white African skunks. Soon after that, the virus etiology of the gripe was established in the USSR (Zil'ber, Smorodintsev, and others) and in other countries. Subsequently, in 1949, Francis and Hedji precipitated another virus of gripe, displaying different antigens and some other characteristics as compared to the first one. The first virus received the name of virus A and the second one -- of virus B.

Pathogenic characteristics. The most susceptible animals are skunks and white mice. Almost all experimental work pertaining to gripe is conducted with these animals. The symptoms of experimental gripe in skunks develop after an intranasal contamination, the incubation period being 2 - 3 days, and they are very similar to human symptoms, i.e. fever, coughing, and nasal discharges. The sickness lasts 7 - 10 days and ends up in recovery, yet, at times the lower section of the respiratory paths are drawn into the infection, resulting in lethal pneumonia. A particular feature of experimental gripe in skunks is its high degree of infectiousness to healthy animals which become stricken even after short contact with sick skunks.

White mice are highly susceptible to the virus of gripe A through intranasal inoculation, and less susceptible to the virus of gripe B. The course of the sickness in mice is monotypical and differs from same in skunks in which the upper respiratory paths are stricken predominantly, while in mice the infection develops in

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the lungs, usually causing pneumonia with the nuclear or draining hepatization of the tissue. The mice become ill 2 - 4 days after inoculation, they become apathetic, the wool is disheveled, respiration is difficult, and death follows within the first 24 hours.

Monkeys are susceptible to gripe, the intratracheal inoculation being the most effective and sometimes resulting in death. In monkeys, the experimentally induced gripe attacks predominantly at the epithelium of the bronchioli.

White rats are much less susceptible to the virus of gripe than skunks and mice. Upon the contamination of the rats with the virulent stem, which was adapted to mice, the virus propagates rapidly in the upper respiratory paths and in the lungs during the first 24 - 48 hours, but subsequently the amount of virus is diminished, so that the reinoculation of the virus from rat to rat, in a series of consecutive passages, brings no positive results. Only mild cases of rhinitis, conjunctivitis, minute nuclear shrinkages in the lungs are manifested, and there are no lethal consequences.

Among other animals susceptible to the virus of gripe, particular attention is given to pigs. This attention is caused by the fact that the gripe, or influenza, in pigs is a spontaneous infection, the inciter of which is a virus acting in association with a hemophilous minute rod (*Haemophilus influenzae suis*). The contamination of pigs with the virus only induces a mild catarrh of the upper respiratory paths. The contamination of pigs with the culture of the hemophilous rod only causes no apparent symptoms at

RESTRICTED

all. However, the combined contamination with both induces a severe, frequently lethal, sickness, accompanied by an inflammation of the upper respiratory paths and pneumonia. Intramuscular inoculation with the virus creates a stable immunity to the subsequent contamination with the virus as well as to the combined virus and hemophilous rod contamination, while the inoculation with the hemophilous rod culture produces no immunity to subsequent virus contamination.

The virus of swine gripe is similar to the virus of human gripe A by its antigenic properties as well as by its capacity to induce experimental infection in swine and man. The stems of the gripe virus, precipitated from humans, will induce in swine, upon intranasal contamination, symptoms analogous with those observed in these animals upon contamination with the swine virus.

Other animals susceptible to artificial contamination with the virus of gripe A are hedgehogs, cats, hamsters, gray rats, certain species of squirrels. Guinea pigs and parrots are widely susceptible.

It was only in rare cases that a gripe infection was reproduced in a human with the aid of an animal-adapted virus. The most convincing tests pertaining to this were conducted by Soviet scientists. Smorodintsev and his collaborators, in 1937, successfully contaminated volunteers with two stems of the gripe virus that was adapted to mice, and established thereby that the failure to become contaminated on the part of some volunteers coincided with a high titration standard of the antibodies present in their blood. There is data available to the effect that most pathogenic

- 283 -
RESTRICTED

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to humans are the freshly precipitated virus stems which were adapted to the chicken embryo only.

Filterability, size, morphology. The virus passes through dark field 1 and 2 filters, Chamberlain L₂ and L₃, and the Zeitz filter. It passes through a 0.2-0.3 µm diameter, and its size, as determined by the Elford method, range from 0 to 120 millimicrons. Other methods, such as centrifuging, electronic microphotography, generally confirmed it. No inclusions were observed in epizoot. The virus was photographed under an electronic microscope and the spherical form of the virus particles was brought to light.

Stability. The virus can be easily preserved in a desiccated state, provided the desiccation was effected at low temperature and in vacuum. Inactivated at an air temperature of 25-30 degrees Centigrade it can be preserved for a month, with, however, a diminution in virulence. At minus 70 degrees Centigrade it will survive for no less than 6 months. When heated to 60 degrees Centigrade and irradiated with ultraviolet rays, it becomes inactivated within 5 - 10 minutes. It is unstable to the effect of formalin, phenol, acids, and alkali. The optimum temperature concentrations for the preservation of the virus are 7.4 - 7.6 °C. $pH = 7.4 - 7.6$.

Cultivation of the virus. The virus was bred in tissue cultures which contained a Tirole solution and tissues of guinea pig and chicken embryos. The most successful cultivation is on a growing chicken embryo. The chorioallantois is contaminated,

- 284 -

RESTRICTED

RESTRICTED

causing visible transmutations on the membrane. Propagation of virus into the allantoic and allantoic chambers is more economical, since under these conditions the virus propagates rapidly, attaining its maximum growth after 48 hours, at an incubation temperature of 36 - 37 degrees Centigrade, and the virus can be obtained in 11 days or less. This rapid and simple method of cultivation furnishes the laboratory with a great quantity of bacteriologically sterile virus containing material.

It is not superfluous to point out that in order to precipitate the virus from the pharynx gargles of grippé-stricken humans, the heretofore prevailing practice of contacting the gurgles (animals hard to obtain) and mice (in the case of which several passages of the virus are required for the reproduction of an infection) has been practically discontinued. Positive results are obtained more often by inoculation into the allantoic or the amniotic chamber of a growing chicken embryo. In this case, it is recommended that the bacteriological sterility of the gurgles is attained not by filtering through filter-candles, but by treating it with penicillin. Various methods for the precipitation of the virus from the gurgles of grippé-stricken patients have been tested for comparative sensitivity. The most rational method turned out to be the introduction of the unfiltered gurgles into the amnion of 11 - 12-day old chicken embryos, a method of higher sensitivity as compared to the precipitation of the virus through passages on skunks.

Differentiation of the virus. As already mentioned, the virus of human grippé has some characteristics in common with the virus of swine influenza. Viruses of grippé A and B are antigenically

RESTRICTED

independent of each other. The immunization of animals with one of the viruses does not lead either to the appearance of antibodies or resistance to the other virus. The virus of gripe A is considerably more pathogenic to laboratory animals, and is accumulated in greater quantities when cultivated on [chicken] embryos. Some structural differences exist between them. By the use of an electronic microscope it was established that the diameter of the virus A particle is 50 millimicrons, and the diameter of the virus B particle is 92.8 millimicrons.

Epidemiology. The sources of dissemination of the infection are the gripe-stricken people, it being the case that those with the light or somewhat obliterated forms of the disease, who are taking it on the go, are the worst offenders. It is predominantly the surface epithelium of the upper respiratory paths that is affected in gripe. This causes the virus to disseminate easily by way of being precipitated from the pharynx during conversation, coughing, sneezing, thereby spreading the infection by the air-drop method. In a typical outbreak of epidemic gripe, morbidity shoots up rapidly, then comes down rapidly, the duration of the epidemic being 1 - 2 weeks, and affecting the susceptible part of the populace. An important factor in the rapid spread of the gripe is the cold season of the year, when the epidemic usually begins and spreads rapidly due to the prevailing maximum crowding of the population. The incubation period does not exceed two days, morbidity lasts 5 - 6 days and, upon recovery, there is a pronounced immunity adequate for the prevention of reinfection.

An epidemic of gripe rarely involves over 30 percent of the

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population, and the dropping off of morbidity is stimulated to a considerable degree by the rapid increase in post-recovery immunity.

There is a periodicity in the epidemic outbreaks of the gripe, such as the regular recurrence of gripe A at 2 - 3 year intervals. For gripe B this regularity has not yet clearly established. It, however, it does exist, the intervals between the outbreaks are of longer duration (4 years).

In reality the malady of gripe does not completely disappear even during the interepidemic period. With the numbers of immune persons predominating in the population, occurring morbidity runs an atypical course with unclear catarrhal symptoms being lost in the mass of other respiratory maladies and only in individual cases is it identified and determined etiologically.

From time to time the epidemic outbreaks of the gripe assume pandemic proportions and in a short time the malady strikes over vast territories involving millions of people. The last recorded pandemic outbreak, which swept throughout the world and carried off in its wake 20,000,000 people, occurred in 1918. In 1943, an epidemic of gripe, induced by virus A, also assumed large scale proportions, and in a short time involved a number of countries in Europe and America. However, notwithstanding the wide propagation, the course of the malady was mild and non-complicated, with very low mortality.

People of all age groups are subject to the gripe, but maximum morbidity and maximum ~~mortality~~ occur among children. The infection is propagated via lines of transportation in direct ratio with the speed of transportation. The highest morbidity is recorded

RESTRICTED

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for cities, particularly in the thickly populated areas.

The epidemic outbreaks of the gripe occur more frequently during the winter season but are known to occur at all times. All geographical locations are affected and only in the tropics, particularly during a pandemic outbreak, is it at its rarest.

The annual run of the occurring cases of gripe is manifested by various clinical symptoms which are basically similar to those in epidemic gripe A and B, but not linked to them etiologically. They appear most frequently as sporadic cases, at times involving small groups, very rarely affecting larger continents of people in the same locality, and are observed during all seasons of the year. However, the maximum number of cases is recorded during the cold weather and after abrupt temperature changes. These cases, in contrast to epidemic gripe, become known as seasonal, or sporadic, gripe (or febrile seasonal catarrhs of the upper respiratory passages).

Due to the fact that morbidity, even though increasing during the autumn-winter or winter-spring periods, yet is observed throughout the year, it is more proper to use the name of "acute catarrh of the upper respiratory passages".

An acute catarrh rarely breaks out all of a sudden. The malady usually develops gradually, with symptoms of the respiratory passages being affected clearly predominant. These symptoms are expressed in paroxysmal coughing, tonsillitis and pharyngitis, head cold with ample discharges. It must be emphasized that the symptomatology of respiratory catarrhs is much varied and polymorphic

RESTRICTED

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Among the complications known to occur are bronchitis, bronchial pneumonia, and the inflammation of the nostril /?/ chambers.

The etiology of acute catarrhs is still unclarified and non-homogeneous. It is entirely probable that a considerable part is played by the effect of external chilling factors, that is, by the superchilling of the organism preceding the appearance of catarrhal symptoms. These factors affect the physiological activity of the vascular and neural network of the respiratory passages, by the virtue of which the state of normal stability of the mucous membranes is changed, and conditions are created, whereby there is promotion of the pathogenic activity of the various conditionally pathogenic microorganisms usually inhabiting the pharynx. A bacteriological examination in these cases usually brings out the presence of hemolytic streptococcus, pneumococcus, and Pfeiffer rods.

The group of acute catarrhal maladies of the upper respiratory passages is always referred to in foreign literature as the common cold, or infectious head cold. Experimental data, pointing to the virological nature of this catarrh is available. American and British researchers conducted experimental contaminations of humans with gargle filtrates from the pharynx of patients. In this artificial contamination, the incubation period ranged between 24 hours and 6 days, equaling on the average 2 - 3 days. Judging by preliminary experiments with filtering through grade-perforated diaphragms, the sizes of the virus range between 30 and 150 millimicrons. In addition to humans, man-resembling apes are susceptible to this virus. All this data, however, requires additional corroboration.

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The usually favorable prognosis to the contrary notwithstanding, the acute catarrhs of the upper respiratory passages are important, since they are the most frequent cause for the loss of productive capacity of the population. This, specifically, is the area of diagnostic confusion, due to the fact that, under the general diagnosis of gripe, various maladies having nothing in common with gripe are erroneously included.

It must be emphasized that the basic epidemiological difference between the acute catarrhs of the respiratory passages, which form a conglomerate of disorders as yet little known, and the epidemic virological gripe, is the less pronounced contagiousness of the acute catarrhs, which contagiousness in some cases cannot even be detected. There are reasons for the opinion that this category of disorders of the upper respiratory passages is constantly present among the populace, at any rate throughout the temperate zones.

Immunity. Accumulated data points to the fact that in gripe there is an adequately pronounced post-infection as well as inoculative immunity. The state of immunity is accompanied by the development of antibodies, and the high content of the latter in the blood is an index of non-susceptibility to the infection. The antibodies circulating with the blood penetrate onto the surface of the mucous membranes of the upper respiratory passages, stimulating thereby the local protective effect of the antibodies at the very gateways of the infection.

In the phenomena of immunity, a considerable part is played by the antigenic diversity of various virus stems. This is

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characteristic of both virus types, and it has been confirmed by serological investigations. It seems that the apparent short period of immunity in guinea can be explained not only by the existence of various virus types (A and B), but also by the intra-species antigenic mosaic of the virus strains.

The inoculation of animals with the active, as well as inactivated, virus antigen creates a stable immunity to subsequent contaminations with even lethal doses of the virus. As a rule, immunization causes the accumulation of antibodies which are formed in both susceptible and non-susceptible animals.

The possibility of creating inactivating immunity, which has been proved in the case of animals, is now being further exploited for the purpose of developing methods for the immunization of humans. As an antigen for intranasal vaccination, the live virus is used, while in subcutaneous inoculations, the virus, inactivated by mild formalin concentrations, is utilized.

Prophylaxis and therapy. One of the general countermeasures against epidemic gripe is, first of all, the isolation of the patient, as the chief source of propagation of the disease. Other measures such as disinfection and ventilating of the premises are useful to some extent. Disinfection of the air by ultraviolet irradiation or aerosol sprays of the propylene glycol and triethylene glycol type, which can be applied in the case of enclosed premises, still remains an open question. The greatest promise is held out by specific vaccination and persistent research is conducted at present in this direction. A concentrated polyvalent vaccine for subcutaneous injection, prepared from an allantoic culture of viruses A and B,

RESTRICTED

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produced encouraging results in immunization experiments on humans. It is, however, not yet clear, whether it will be suitable for specific mass prophylaxis.

Harodintsev recommends the intranasal introduction of an immune nasal spray. This method is defective on account of the rapidly disappearing passive immunity.

Prophylactic measures against secondary bacterial complications in gripe call for the use of sulfa drugs, particularly sulfadiazine.

There are no effective therapeutic means that can be used in gripe.

Laboratory diagnosis. To date, there are no reliable methods for the early diagnosis of the gripe. Laboratory diagnosis is accomplished by introducing samples from the pharynx of patients into susceptible animals or into a developing chicken embryo, with subsequent precipitation of the virus. Recently, the test for the agglutination of erythrocytes (hemagglutination) and the complement fixation test have been employed diagnostically in searching for the virus antigen. The above reactions are in a stage of experimental development and are calculated to be effective in early laboratory diagnosis, while the precipitation of the virus from patients, being a lengthy procedure, constitutes, together with investigating the accumulation of antibodies in the blood of convalescents, the method for a precise but retrospective diagnosis.

The precipitation of the virus from the patient is

RESTRICTED

affected during the first days of the malady. The material for the inoculation of animals is the pharynx gargle taken from a patient the first or second day of the sickness. The patient is made to gargle his pharynx with 15 - 20 cubic centimeters of a physiological solution, pure or with a 10 percent normal serum. After a thorough gargling, the liquid is collected into glass tubes or small jars containing strike up beads. The nasal passages of the patient are then swabbed with a cotton tampon, and the tampon immersed in the gargle. The liquid is to be put to use as fast as possible, otherwise it should be kept in the refrigerator.

Before using it on animals, the gargle liquid is centrifuged for the precipitation of large particles, and filtered through colloidal or asbestos plates. Since in filtering, particularly through asbestos plates, part of the virus is lost, they use the non-filtered gargle, to which it is recommended to add a solution of sulfadiazine or penicillin (150-200 moles per 1 cubic centimeter). [The Russian text uses "OE", which might mean "volumetric units" in the place of moles/]. These solutions are used as bacteriostatic substances, which do not affect the virus, but impede the development of sporadic bacteria present in the gargle.

White African shunks, white mice, and white rats are used for the precipitation of the virus. Under light ether narcosis, these animals are contaminated intranasally with the above pharynx gargles taken from patients. In order to adapt the virus from the gargles to the organism of laboratory animals, they use serial passages, the material for which

-278-
RESTRICTED

RESTRICTED

are the secretions of the mucous membrane of the nose (skunks and bats), or the lungs of an animal put to death (a mouse). When the virus is precipitated via the contamination of chicken embryos, the methods used are the inoculation of the material onto the corioallantoic membrane, into the allantoic or amniotic cavity.

To identify the virus in the allantoic fluid or in the lungs of laboratory animals it is necessary to determine its specific characteristics. For this purpose, tests are made for filtration and subsequent contamination of susceptible organisms and embryos, for determining the ability to agglutinate the erythrocytes in chickens, dogs, guinea pigs, for determining the ability of specific immune sera to retard the reaction of hemagglutination and to neutralize the virus in neutralization tests, during the contamination of white mice and embryos.

The hemagglutination test for the detection of the antigen in the gargles of gripple patients is used in laboratory diagnostics for epidemic gripple, and is based on the principle of the adsorption of the virus by the erythrocytes. The patient gargles his throat with a sterile physiological solution, then the liquid is collected into glass tubes for settling, after which only the top clarified layer is taken for the test. To 0.5 cubic centimeter of the gargle in a clean glass tube, 0.5 cubic centimeter of the gargle in a clean glass tube, 0.5 cubic centimeter of 0.5 percent suspension of erythrocytes of a guinea pig is added. The mixture is shaken thoroughly, and left to settle for one hour at room temperature. For

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control purposes, 0.5 cubic centimeter of physiological solution is added to 0.5 cubic centimeter of erythrocytes kept in another tube. The result is determined by the nature of the precipitate. If the triple antigen is present in the gargle, the erythrocytes fall out in the form of a flocculent lace-
 -ed precipitate. When the result of the test is negative, there is a firm compact precipitate of erythrocytes in the form of round disks with sharply outlined edges. Such a precipitate forms also in the control test tube, where the erythrocytes are mixed with a physiological solution.

In the presence of agglutination, the latter process is tested specifically for retardation by the use of an anti-
 -triple serum. For this purpose, anti-triple immune sera A and B, in 4 dilutions (1:5, 1:20, 1:40, 1:80), 0.5 cubic centimeter each, are poured into test tubes, into which 0.25 cubic centimeter of the gargle and 0.5 cubic centimeter of a 0.5 percent suspension of erythrocytes of a guinea pig are added. The mixture is shaken up thoroughly, allowed to settle for an hour at room temperature, and results registered. Control tests for the spontaneous agglutination of erythrocytes are conducted with normal serum in the same dilutions.

The result of the test for specific characteristics is considered positive in the case when, in the presence of agglutination in the control test tubes with normal serum, the specific characteristics are absent in the test tubes with immune sera, as well as in the test tube containing the physiological solution (Peterson).

For the same purpose, the simpler method by Shubladze

225

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and Solov'yev, consisting of the reaction of agglutination on a microscope slide.

The diagnosis of grippe by discovering the virus in the respiratory passages of patients with the aid of the complement fixation test is conducted by a method developed by Peterson. The antigen compound is the pharynx gargle, which is clarified by centrifuging and taken in dilutions, which will not produce self-inhibition (1:10, 1:20). The test is conducted with an immune anti-grippe serum in accordance with the method described on page 77 of the original text. This research, however, did not find any practical application, and, in order to search out the antigens in the organism of the patients with the aid of the complement fixation test, additional investigations will have to be conducted.

For the retrospective diagnosis of epidemic grippe, the tests of neutralization, complement fixation, and hemagglutination inhibition are used. The reaction of neutralizing the virus with immune sera is based on the ability of the antibodies in the sera of convalescents to specifically inactivate the virus, so that the mixture of the virus and the serum will not induce the sickness in experimental animals. The procedure is to mix the serum with a steady dose of the virus, known as priori to be lethal to a mouse (for instance, 1000 minimum lethal doses), or the mixing of a steady dose of the serum with various dilutions of the virus. Results are judged by the degree to which the lung tissues in the experimental mice were affected, or by the mortality of the mice during

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10 days of observation after the intranasal introduction of the mixture. Before use, the mixture is kept in a thermostat at 37 degrees Centigrade for 30 minutes. The mixture is introduced intranasally, while the mice are under a mild ether narcosis, in a quantity of 0.05 cubic centimeter, it being the case that, for each dilution, 4 mice of equal weight are taken, with the corresponding number of control mice, inoculated with the virus in a mixture with a normal serum. The neutralizing activity of the serum is determined by the number of non-stricken mice as compared to the number of control mice.

In the complement fixation test conducted by the Martinsson method, a 10 percent suspension of hepatized lungs of contaminated mice, prepared in a physiological solution, is used as the antigen. This suspension is stored at minus 4 degrees Centigrade over a period of 2 - 3 weeks, without losing its active characteristics. As needed, the suspension is thawed out and centrifuged for 15 minutes at 1500 RPM. The transparent liquid above the precipitate is diluted to half its concentration and used as an antigen. The sera are inactivated at 58 degrees Centigrade for 20 minutes. The complement and hemolytic system are prepared in the usual manner. The procedure in this case differs basically from the one described before in that the fixation of the complement in this case takes place in the thermostat at 37 degrees Centigrade in the course of one hour. The registration of the test is effected by an additional hour of aging at room temperature.

The Virus of Psittacosis.

- 297 - RESTRICTED

RESTRICTED

General data. Psittacosis is a severe ailment of parrots and is manifested in the form of a generalized infection, with intestinal phenomena predominating. These birds are the specific source of epidemics in humans in Europe and North and South America. In humans the salady has a typhoid character, with pulmonary complications in most cases. Following a 4 to 15 days incubation period, a general indisposition, a sensation of being broken up, headache and nausea set in. In individual cases there are nasal hemorrhages, diarrhea, vomiting. The temperature rapidly rises to 39 - 40 degrees Centigrade and stays at this level for the remainder of the morbid period, which is accompanied by delirium. At times, already on the day following the first symptoms, there is a severe inflammation of the lungs leading to the complete hepatization of the latter.

Alongside of severe cases, there are diluted, remittent forms of the salady, with the temperature not exceeding 38 - 39 degrees Centigrade, and only general symptoms and mild pulmonary lesions. Such mild cases of psittacosis usually occur in young people, they run their course in 8 - 10 days, ending in recovery. Mortality in human psittacosis is high, and, in accordance with various data, it ranges between 20 and 45 percent. The most characteristic feature revealed by pathologic-anatomical examination is the inflammation of the lungs. Frequently, pneumonia is accompanied by mucopurulent bronchitis. Pulmonary transmutation occurs on account of thrombosis and vesicular hemorrhagic desquamative pneumonia, complicated by secondary bacterial infection.

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macroscopically - the lung becomes red, with nuclei of hepatization. Microscopic examination reveals proliferation nuclei in the alveolar epithelium, the alveolar spaces are filled, at first, with serous, and then, with fibrinous exudate, with leukocytic infiltration. In addition to the lesions of the lungs and pleura, there is hyperemia of the brain and spinal cord. Parenchymatous regeneration is especially noticeable in the kidneys and liver.

Inoculation of the virus. In 1930, Reason, Western, and Simpson proved the existence of a filterable virus in the organism of stricken parrots, and in substances taken from stricken humans. From the blood, serum, and lung exudate they produced virus strains, pathogenic to laboratory animals.

One of the main characteristics. Psittacosis, as already mentioned above, is a natural enemy of parrots. Parrots of different varieties are the source of infection in America as well as in Europe. Spontaneous sickness occurs not only in parrots, but in other birds too: storm petrels, pigeons, it being the case that the majority of adult birds, even though contaminated, appear healthy. The symptoms of the disease come to light principally in the case of young birds, and are manifested by lack of appetite and adynamia. With the subsequent development of the disease appear ample nasal discharges, asthma, coughing, gastrintestinal disorders accompanied by a malodorous green stool. Death occurs in 8-9 days. In some cases the disease assumes a chronic form, in some cases it is abortive, ending in recovery.

The artificial contamination of parrots in the laboratory is easily successful through the intramuscular inoculation,

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as well as by the introduction of the virus through the mouth and nose. Canaries, chaffinches, and Bengals also turned out to be susceptible. Chickens are less susceptible. Reaffinches and Thrushes are, seemingly, non-susceptible. Harvill and his collaborators were the first ones to report the susceptibility of mice and to show that the virus can be passed through these animals. With the aid of an intraperitoneal contamination of mice it is possible to precipitate the virus from the tissues of a stricken mouse. This method was utilized in the diagnosis of the disease. Series of passages through mice were obtained via intraperitoneal inoculation with a suspension from the liver and spleen of infected animals.

The introduction of the virus into the brain of mice induces encephalitis and the subsequent recovery, by the use of this brain, makes for the unlimited preservation of the virus. Guinea pigs and rabbits are less susceptible than mice, but the intracerebral contamination is still the best method. Intracerebral inoculation of guinea pigs induces the reddening, swelling, and the formation of papules on the skin. This skin reaction of the guinea pig is utilized as a method for virus titration.

In monkeys, contaminated via the trachea and nose, occurs the development of pneumonia, similar to the one observed in humans. The intracerebral inoculation induces in monkeys a non-lethal form of encephalitis.

Filterability. Size. morphology. The virus of psittacosis passes through the Berkefeld V, Chamberland L₁, L₂, L₃ filters, and occasionally through Zeyts filterplates. Being

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the largest virus, it occupies the middle place among the typical representatives of the small viruses, on the one hand, and the rickettsia, on the other hand. By way of fractionation centrifuging at 5000 r.p.m. for a period of 2 hours, the virus is concentrated and purified. The virulent material of various origin, such as from the liver and the pericardium of parrots, from the alveoli of human lungs, from the spleen and the cerebral membranes of a mouse, shows the presence of elementary corpuscles, which are the inciters of the disease. They are minute round, or somewhat oval, formations which show up in the smear singly, in pairs, or in irregular clusters. Their sizes, as determined by ultrafiltration, oscillate between 230 and 330 millimicrons. In microsections and microimpressions the corpuscles are invariably deposited within the cells of reticular endothelium, while cells are often literally packed with them. On smears, extracellular forms may be discovered as a result of the crushing of a cell. They can be easily stained as Gram-negative bacteria or rickettsia. In accordance with the Romanovsky - Giemsa method, after differentiation, in a solution of orange and tannin, the elementary corpuscles assume a purple hue. They can be easily stained by Leffler or any other polychromatic blue, also by the Fastan method.

Stability. The virus will survive in a phosphate buffer solution ($\text{pH} = 7.6$) at 4 degrees Centigrade for one month, and in a desiccated and frozen state for over 270 days. The virus will disintegrate within 30 minutes by being heated in a water bath at 80 degrees; at 55 degrees it is inactivated, not

- 501 -

RESTRICTED

RESTRICTED

always completely. A 0.5 percent formal solution at 37 degrees centigrade has no effect upon the virus during the first 30 minutes. A 0.5 percent formalin solution inactivates the virus without affecting its immunogenic properties. Ether in a 10 percent concentration somewhat weakens the virus of psittacosis. It is also weakened under the effect of penicillin.

Virus cultivation. The virus of psittacosis can be easily cultivated on tissues and on the chorioallantois of a chicken embryo. When cultivated in an embryo, it can be demonstrated in the chorioallantois under the microscope, due to the great numbers of elementary corpuscles in the tissue. The contamination of the embryos into the yolk sac is still more effective, and by it greater numbers of virus may be obtained.

Differentiation of the virus. By its morphology and antigenic properties, the virus of psittacosis is similar to the virus of meningoencephalitis of mice, venereal lymphogranuloma, trachoma. This is the basis for uniting the above enumerated viruses into one group.

By immunizing mice with a formalin-inactivated virus, it is possible to obtain an immune serum, which is capable of neutralizing the virus. The mild neutralizing capacity of the serum may be discovered in the tests of inoculating the mixture of the virus and the serum into the abdominal chamber of mice. Neutralization is manifested more clearly, if various dilutions of this mixture are vaccinated into the skin of a guinea pig. Neutral mixtures cause no reactions on the skin, while the

RESTRICTED

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virus in mixture with normal serum induces reddening and the formation of specific papules. Guinea sera of guinea pigs and mice are capable of agglutination, the washed off elementary components of psittacosis. This test is specific. The complement fixation test was also developed, with a suspension from the spleen of contaminated mice used as an antigen. The application of this test brought out the presence of two antigenic groups of the virus: thermostable and thermolabile. Each one of these groups induces the formation of specific antibodies in immunized animals.

Epidemiology. The contamination of a human is due mostly to direct contact with stricken birds and their secretions. It is an established fact that the malady strikes mostly in adults. There are cases on record, in which contamination stemmed from a human being, such as 40 cases of the malady occurring among hospital personnel, where contact with birds was completely absent. The origin of epidemic outbreaks is linked with the existence of stricken birds or birds that are virus carriers. It is an established fact that there are many cases of latent infection present in parrot nurseries.

Immunity, Prophylaxis, and therapy. Considering the rare occurrence of the disease in adolescents and its complete absence among little children, it may be assumed that the above groups possess natural immunity. In adults, who recovered from the disease, only single cases of recurrence are on record. Antibodies, which are neutralizing the virus of psittacosis, were found in humans rarely and in small amounts. As yet,

RESTRICTED

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there are no adequate prophylactic and therapeutic methods in relation to miltacosis. Serum prepared from recovered ascents are used for sero-therapeutics. There are some indications that sulfamide compounds and penicillin have a certain therapeutic effect in miltacosis.

4. Infectious Viruses.

Into this group we conditionally relate the inciters of virus diseases, in which no single organs are affected selectively (such as Dengue fever, Japanese encephalitis), or in which there is a diffusion of infection (such as yellow fever, measles) and the general circulation of the virus in the blood stream. Also here, we furnish data on the viruses striking selectively at the lymphatic system (such as mumps, venereal lymphogranuloma).

At this point it is considered unnecessary to elaborate in detail on the virus of infectious hepatitis, since this disease was insufficiently studied, even though its clinical manifestations are well known. We, therefore, limit ourselves to some brief data on this malady.

Infectious hepatitis, virus jaundice or the Botkin malady, which was segregated by Botkin in 1891 into an independent nosological unit, attracted particular attention during the war. There are two forms of virus infection jaundice. The first form - serous jaundice, which is induced by vaccinations of virus containing homologous sera, accidentally taken from a sick human, or a virus carrier. Such cases were observed at times during the inoculation of children with anti-rubeola

- 304 -
RESTRICTED

RESTRICTED

sera, during blood transfusions, and during prophylactic vaccination with various vaccines. As an example, can be cited the case of the vaccination of 1,000 American soldiers with anti-yellow fever vaccine, containing human serum. The serum turned out to be contaminated with the virus of jaundice, which led to the development of hepatitis in 24,000 soldiers.

Another form of infectious hepatitis develops epidemically, with a display of analogous clinical symptoms, the only difference consisting in that the incubation period in serous jaundice may fluctuate between 50 and 137 days, while in epidemic infectious hepatitis it ranges from 15 to 34 days.

As a rule, the disease comes into the open suddenly and is accompanied by fever, chills, and rapidly passing headaches of medium severity. At the beginning, the typical symptoms are displayed by the upper respiratory passages (cough, catarrh of the nasal mucous membrane, laryngitis and pharyngitis), and also gastrointestinal disorders. During the first phase, the liver is not enlarged, but there is some pain in the area of the liver. Such a state continues for 3 - 4 days, and is followed by nausea, loss of appetite, vomiting. This is when the liver becomes enlarged and more painful. At the height of all these symptoms occurs the development of jaundice. In some patients there is no pre-jaundice stage, and in such cases the initial symptom is a slight yellow tinge of the sclera and skin.

The virusological nature of infectious hepatitis has been proved by numerous tests of artificial transmission of

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the malady from a sick to a healthy person. The contagion is successful whether by the introduction of the infectious material through the mouth or parenterally. The virus was located in the patients' blood, intestinal contents, and in the pharynx. The virus is capable of sustaining a temperature of 56 degrees Centigrade for 30 minutes, and will pass through a ^{Seitz} Zerk filter. To date, no animals susceptible to this virus were discovered, and attempts at cultivating the virus on tissues failed.

The pattern of propagation of epidemic hepatitis remains obscure. It is possible that the infection is transmitted through the feces of patients and through the air in the upper respiratory passages. The factor in the transmission of the disease may be, in addition to direct contact, also water and food. The maximum number of cases is observed in September - December.

HEMORRHAGIC FEVER

At this point it is ~~in place~~ to mention the Crimean Hemorrhagic fever, a virus disease encountered in the steppes of the Crimea during spring-summer months. This malady was described by Chumakov and his collaborators (1944 - 1946).

Hemorrhagic fever is characterized by a sudden rise in temperature, head and muscle aches, and the frequent development of hemorrhagic symptoms, such as rashes, skin hemorrhages, bleeding of the gums, and, in severe cases, bloody vomiting, and diapedetic hemorrhages. The virus of hemorrhagic fever is found in the blood of the patients during the first 3 - 5 days, it is filterable through Berkefeld V, N candles, ^{Seitz plates [discs?]} Zerk diaphragms, and can be preserved for long periods in vacuum degassed serum.

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Cats, dogs, guinea pigs, etc. are susceptible in a rather unusual manner, and it is the opinion of the authors that only humans are completely susceptible to the virus of hemorrhagic fever. The virus carriers are the striped ticks *Yulionus sanguinolentus*.

The Virus of Rubella (Measles).

General data. Rubella is an acute infectious disease with an incubation period of 1-12 days, with the subsequent development of a catarrhal phase, characterized by conjunctivitis and the inflammatory condition of the mucous membranes of the pharynx and the upper respiratory passages. At the beginning of this stage, peculiar formations, known as the "Koplik" spots, appear on the inner surface of the lips and cheeks, opposite the molars. Minute, the size of a pinhead, yellowish-white, or bluish, they protrude slightly from the surface of the mucous membrane and are surrounded by a small zone of hyperemia. Subsequently, a maculo-papular rubular rash, first appearing on the mucous membrane surfaces of the mouth and throat, spreads to the skin of the face, head, then covers the neck, body, and extremities.

The catarrhal phase lasts, on the average, 3 - 5 days and is accompanied by a rise in temperature to 38-40 degrees Centigrade. Then, within 1 - 2 days, there is recovery. However, frequently there are severe complications, due to secondary bacterial infection, which may lead to death after a week or even a month from the time of disappearance of the rubular rash. The predominant complications are bronchial pneumonias, inflammation of the middle ear, mastoiditis^{es}, sinusitis^{es}, enteritis^{es},

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and, frequently, tubercular encephalitis. Measles is predominantly a childhood disease, and in 80-90 percent of all cases it strikes at children up to the age of 6. Patho-
biological-anatomical transmutations in measles are not specific.

Virus precipitation. The virusological nature of measles was established by the experimental transmission of the infection via the introduction of a patient's blood into the body of a healthy human, and also by contamination; monkeys with a virus filtrate from a human being. The secretions from the pharynx in the early stages of the disease also contain the virus.

Pathogenic characteristics. Monkeys are susceptible to this virus, with the introduction of the virus by intra-rachal inoculation being the most effective. Monkeys become ill when inoculated in this manner with the filtered or non-filtered filtrate from the pharynx of patients, taken within 12 hours from the appearance of the tubercular rash, invariably become stricken with the disease. The course of the disease in monkeys is very similar to that in humans. The malady was then transmitted from the stricken monkeys to healthy ones by intra-rachal inoculation of a suspension from the mucous membrane or the skin of monkeys that were killed on the 2 - 6th day of the development of clinical symptoms. The blood of the monkeys also contained the virus.

Experimental measles was reproduced in monkeys by a number of virusologists, particularly by Smirnov, Konova, and Ryasantseva (1939). Clinically speaking, the malady in monkeys runs a little wilder course than in children, the incubation

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period of up to 6 to 16 days. Results, according to some data, can be contaminated with the virus of measles, but the only symptom they show is a rise in body temperature. All attempts to infect cynomolgus and white rats failed. In white mice, it is possible to induce a symptomless measles infection, which can only be detected by a complement fixation test conducted under critical temperature conditions. (Jette and others, 1960).

Filterability, morphology. The blood of measles patients is still contaminated after having been filtered through a Zeta filter and Berkefeld candles. A pharynx sample from patients, after being filtered through Berkefeld candles, also retains its infectious characteristics. Elementary corpuscles were discovered in the cells of the pharynx, tonsils, spleen, in the lymph nodes, and in the leucocytes of the blood. All these discoveries, however, require additional research and confirmation.

Stability of the virus. The virus in the blood and in the pharynx sample can be preserved at zero Centigrade for several days, and, with the addition of 50 percent glycerin, for 3 months. Subcellular material, after having been vacuum desiccated, retains its contaminative capacity for a long time. At room temperature the virus is inactivated after 24 hours, a water bath of 56 degrees Centigrade will disintegrate it in 15 minutes, a 10 percent ether solution inactivates the virus in 40 minutes.

Cultivation of the virus. The virus of measles can be bred in tissue cultures, consisting of a Tirode solution, monkey serum, embryonic tissue of a chicken embryo, chick plasma, and

RESTRICTED

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also as the inoculo-antisera of a chicken or rooster. The last
method was successfully used by Shimizu and Konova in 1930.

Epidemiology. Measles is known throughout the world.
epidemic outbreaks of various intensity are observed in various
countries, year in, year out, and they are basically linked to
the appearance of a new susceptible section of the population,
that is the present new generation. Due to the fact that the
great majority of the adult population have already had measles,
the epidemics as well as the sporadic cases are predominantly
generated among children.

Measles is a drip infection that enters through the
upper respiratory passages. The only source of infection
in measles is the measles patient. Letality usually fluctuates
between 0.2 and 1.4 percent and above. Frequently, measles is
accompanied by complications, linked to secondary bacterial in-
fection, resulting in higher mortality.

Immunity, prophylaxis, and therapeutics. There is no
inborn natural immunity to measles. Usually, after six months
from birth, children become susceptible to measles. During the
first months of life, the immunity of the baby is a passive one,
having been acquired through the presence of immune bodies trans-
ferred from the mother's body via the placenta. An immunity ac-
quired after having endured the sickness, persists through life,
as witnessed by the antibodies found in the organism of almost
all the people, who have had measles.

Some effective means for the specific prevention of

310
RESTRICTED

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measles and pertussis, its course and effect are known. They are the prophylactic inoculation with the serum from convalescents, the serum from adult or immune individuals, obtained from extracts of human placenta. The passive immunity acquired after inoculation with such preparations lasts several weeks and is usually adequate for the prevention of morbidity in children, who were in contact with infectious patients. These preparations are to be inoculated intramuscularly, and, if possible, immediately upon the contact of the healthy child with a sickened child. The degree of the placenta extract, or the serum from convalescents, or the blood serum of other children depend on a series of factors, such as pattern of the preparation, time of inoculation, age and weight of the child, and, possibly, the length of the contact period. The gamma-globulin fraction of the serum, containing the measles antibodies in a state of concentration 20-30 times over, recently found successful application.

Laboratory diagnostics. As yet, there are no readiness of laboratory diagnostics suitable for practical application. The diagnosis is made on the basis of a clinical examination of the patient. As to the agglutination test of the virus-loaded bacteria (Serjegov, Vainina, Myazantskaya), its practical applicability is, as yet, in the stage of experimental development.

The Virus of parotitis (Mumps, Epidemic Parotitis, Inflammation of the parotid glands)

General data. The mumps, or epidemic parotitis, is an acute infectious disease, with an incubation period between 8 and 35 days (18 days on the average), characterized by the in-

RESTRICTED

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Inflammation of one or both parotid glands, mild pharyngitis, fever, headache, general malaise. The virus disseminated through the bloodstream, may become contained in various organs and produce histiolytic afflictions, such as orchitis, epididymo-orchitis (which, during the period of puberty, develops in 1 percent of all cases), inflammation of the salivary glands, or, sometimes, the pancreas, encephalitis developing in 10 percent of cases of mumps-stricken adults). The disease strikes predominantly in children of the 5 to 15 age, and is rarely fatal. The virus can be discovered in the saliva of patients only during the first 3-4 of the malady.

Precipitation of the virus. The virological nature of mumps was established in 1934 by Wond and Jeonpaster, who induced a typical case of parotitis in monkeys by introducing into the Steno's duct (the duct of the parotid gland) of the latter the saliva filtrate from a patient. These investigators made 10 consecutive passages of the virus on monkeys, and with the virus from the 11th passage they infected a human with parotitis. Many investigators then reported the precipitation of the virus of parotitis by similar methods in monkeys.

Pathogenic characteristics. Various species of monkeys (Macaca- species and, particularly, the panzee) are susceptible to the introduction of the virus into the parotid gland, and become stricken with a typical clinical case of inflammation of the salivary glands similar to human mumps. It is also possible to induce parotitis in monkeys by a simultaneous intraabdominal and intravenous inoculations with the virus. Intracerebral inoculation of these animals with the virus of mumps induces

RESTRICTED

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encephalitis, and death usually follows in the first day after the inoculation.

Among other animals, rats are the most sensitive to the introduction of the virus into the salivary gland and into the brain. Rabbits, guinea pigs, mice, and dogs are not susceptible to the virus of a lethal parotitis.

Pathogenicity of the virus. The virus passes through *Yersinia* and *Escherichia coli*. No reliable proof of the existence of elementary body stages is available as yet.

Stability of the virus. The virus survives in suspension for 2-4 weeks, and in a refrigerator and vacuum-sealed state - several months. Ultraviolet light and formalin solution disintegrate the virus within an hour.

Cultivation of the virus. The virus can be cultivated on the chorioallantoic membrane of a chicken embryo, but its propagation is in a stage of independence active. A more successful cultivation of the virus can be effected by contaminating the chicken embryo into the yolk sac. The cultivation of the virus by a series of passages into the amniotic chamber of the chicken embryo turns out to be the most effective. The introduction of the infected amniotic fluid, in a 10^{-6} dilution, caused an infection in monkeys.

It was also established that the consecutive passages of the virus by the use of this method cause a modification in its characteristics; the virus becomes little pathogenic to monkeys,

RESTRICTED

SECRET

acquires the ability to split into the erythrocytes of chickens. This acquired capacity for hemagglutination can be neutralized by the sera from neonatal chicks and recovered. However, the virus, after having made 25 passages on eggs [see also 1953], was unable to induce an infection in chickens, and actually vaccinated these animals against the subsequent contamination with an active virus. This data led to the idea of utilizing, by analogy with yellow fever vaccine, for the active prophylaxis of humans against mumps as a parotid vaccine, the virus of parotitis bred on a growing chicken embryo. This method of cultivating the virus on eggs is now used for the detection of the virus and its precipitation from patients.

Epidemiology. Epidemic parotitis may occur anywhere, and it may strike at people of any age, with most cases, however, occurring among children in the age group 5 - 15. The infection spreads by the droplet route, due to the presence of the virus in the saliva during the first days of the disease.

Immunity, Prophylaxis, and Cure. It seems that natural immunity does not exist, since most people upon contact with patients, usually are stricken with the malady. The immunity acquired after having endured the sickness, usually lasts through life. There are, however, rare cases of recurrence. The sickness induces the production of specific antibodies in humans and in ~~antisera~~ ^{monkeys}. The antibodies are discovered by the complement fixation test, which is accompanied by hyper-sensitivity of the skin, determinable by an intradermal test with the antigen obtained from the infected parotid gland of monkeys. The possibility of cultivating the virus in a growing chicken embryo

SECRET

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developed a new method for the obtaining of the active antigen.

Live vaccine injections of the formalin-inactivated virus increase an active immunity in 94-95%.

For prophylactic purposes, in the case of humans, intramuscular inoculations with serum from convalescents in the amount of 3 - 10 cubic centimeters, within the first 7 - 10 days upon contact, are resorted to. In the case of adults, larger dosages of the serum are to be used. In the absence of serum from convalescents, the serum of adults, who have endured the disease in childhood, may be used. But since the content of antibodies in such serum is lower than in the serum of convalescents, the dosage should be at least doubled. A live vaccine, prepared from a virus, that has undergone modifications through its cultivation on tissue culture embryos, is now being tested for prophylactic use in groups.

For therapeutic purposes, intramuscular injections of 20 - 25 cubic centimeters of serum from convalescents, or of 50 - 100 cubic centimeters of serum from adults, may be used. There is, however, no proof of the effectiveness of such sera in reducing the mortality and the encephalitis [which sometimes develop in adults stricken with yellow fever].

The virus of Yellow Fever.

General data. Yellow fever is an acute infectious disease, characterized in typical cases by a sudden start, fever, jaundice, albuminuria, hemorrhagia, vomit, prostration. Patients suffer from intense headaches, pains in the occiput and backaches. The

RESTRICTED

face of the patient is covered, with sharp hyperemia of the mucous surfaces of the eyes and mouth. The tongue is spotted red. Gastrointestinal disorders are also registered. Jaundice appears on the 3 - 4th day, the tongue assumes a brown tinge and becomes dry. At this stage, the condition of the patient improves in some cases, and the disease may even be terminated, but more often than not the temperature goes up again, bringing on a general state of feverishness, which is accompanied by the rejection of a brown-black vomitus, induced by gastrointestinal hemorrhages and bleeding from the gums and nose. Beginning with the 3 - 4th day of the malady, the amount of albumin in the urine is on the increase.

The incubation period usually amounts to 3 - 6 days, in some cases up to 10 days. The period of convalescence sets in 1 - 2 weeks after the beginning of the sickness, and a state of health is gradually restored. In severe cases, death occurs on the 6 - 8th day from the beginning of the malady. Mortality oscillates within a wide range, from 10 to 45 percent and above.

A pathologico-anatomical examination [autopsy] discloses the following: saturated jaundice coloration of the intestines, sharp hyperemia of the mucous and serous membranes, spotted yellow color of the liver and the kidneys. The most typical micro-transmutations become evident in the liver. They are manifested by adipose regeneration and necroses. In the kidneys, there is adipose regeneration and necrosis of the epithelial cells of the ducts. The cardiac muscle also shows adipose regeneration.

RESTRICTED

Filter proof studies. In 1926, Walter Reed and Carroll A. Hooper first tried to prove that the virus of yellow fever will pass through Chamberlain filter-bundles, and that a filtrate can be infective to the filtrate. This discovery was fully confirmed in 1927 through the investigations conducted by Stokes, Taylor, and Johnson, who successfully reproduced experimental yellow fever in guinea pigs and monkeys, and established the possibility for propagating the virus on these animals. The virus in monkeys, as is the case in humans, is of the viscerotropic characteristic. It was subsequently discovered that mice are susceptible to the virus through intracerebral inoculation, and that, after a series of consecutive passages on mice, the virus will lose its viscerotropic characteristics and acquire neurotropic characteristics.

Pathogenic qualities. The virus of yellow fever is viscerotropic and neurotropic. These characteristics vary within wide ranges. If individual stems of the virus naturally possess both characteristics, they are called the pantropic virus stems of yellow fever. If such a pantropic virus stem is used to effect several passages on mice, via intracerebral inoculation, it loses completely its viscerotropic characteristics, or the ability to strike at the internal organs of a mouse, such as the liver, heart, lungs, and kidneys. At the same time, it propagates well in the brain of these animals, inducing encephalitis. Monkeys, mice, and hedgehogs are susceptible to the virus via extraneural and intracerebral inoculations. Bats can be contaminated intracerebrally. When monkeys are inoculated intracerebrally with the neurotropic virus stem, encephalitis follows. If the pantropic virus stem is introduced by the same route, it results in yellow

RESTRICTED

fever, with infections of the liver, heart, spleen, lungs, and also, including cats, guinea pigs, white rats, shrews, guinea pigs, resistant to the inoculation with both the neurotropic and viscerotropic viruses of yellow fever.

Filterability, size, morphology. The virus of yellow fever passes the 100 mμ sieve, 1, 100 mμ sieve, 100 mμ sieve. The virus size was established by filtration through membrane-filtered diaphragms of varying pore size 10 and 27 mμ. The typical morphological indicator of the infection is the presence of oxysphule intranuclear inclusions in the liver cells, which can be detected regularly in experimentally contaminated monkeys, and less regularly in human series, when autopsies are performed.

Stability of the virus. The virus is maintained in the laboratory in concentrations of 10⁶ of the hepatic or cerebral tissue of monkeys or on mice. It can survive for 100 days in 50 percent glycerol at zero Centigrade. In a vacuum-sealed state, it can survive for over 2 years. The virus will disintegrate at high temperatures: boiling in a water bath at 65 degrees Centigrade disintegrates it within 10 minutes. The photodynamic action of actinylene blue becomes lethal within 15 - 20 minutes. A 5 percent formalin solution at 30 degrees, a 16.7 percent of ethyl alcohol solution and a 0.3 percent phenol solution will disintegrate it within 30 minutes. A 0.1 percent formalin solution will rapidly kill the virus at room temperature, and will take 48 hours to kill it at zero Centigrade. An acid medium (pH=3-5) inactivates the virus within three hours.

RESTRICTED

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Cultivation. The virus of yellow fever can be bred successfully on tissue cultures such as monkey kidney cells. After prolonged passages in such cultures the highly virulent embryonic virus strains, in most cases, change their pathogenic characteristic, retaining, however, their antigenic power, which is expressed in the ability to produce specific antibodies, - - - - - and their stable infectivity to animals. All these cultures are maintained at 37 degrees centigrade, and the transplantings were collected every 2 - 4 days. The virus is also cultivated on the embryallantois of a chicken embryo.

Dissemination of the virus. There are 4 antigenic differences between strains of various localities. The specific characteristics of the master of yellow fever, is contradistinguished from other strains, a result of which is neutralization of the virus with sera from convalescents, or the complement fixation test, and cross-reaction tests on animals.

Epidemiology. Yellow fever became well known with the discovery of America by Columbus. In addition to South America, the disease is widespread in Africa and Western India.

The Murchley theory on the dissemination of the disease by mosquitoes, which theory was advanced as far back as 1811, was confirmed only 20 years later, when it was established that the mosquitoes *Aedes aegypti*, known before under the name of *Stegomyia fasciata*, or *calopus*, are the carriers of yellow fever. The virus circulates for the first 3 days in the blood of the stricken human. An interval of 12 days must pass, before the mosquito, having obtained his fill from the blood of the stricken human,

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will be capable of transmitting the infection through its sting. If the infective mosquitoes are maintained at 37 degrees Centigrade, the virus will propagate more rapidly in the body of the insect, and its sting may be regarded as infectious within several days. The mosquito, as an agent, is a house mosquito exclusively, and its larvae are usually reared in small stagnant ponds. In cities and rural areas, yellow fever is transmitted from person to person exclusively by the mosquito *Aedes triseriatus*.

It has been established experimentally that other species of mosquitoes may serve as carriers under artificially created conditions. It is not known, however, whether they have any epidemiological significance.

Yellow fever, alongside of malaria, has been for a long time the most widespread disease in South America and Africa. It has been noted that in cities, once known for their long periods of mortality, there are present great numbers of immune people, with the sickness gradually disappearing, and rare outbreaks occurring principally among the non-immune newcomers. The outbreaks are frequently so mild that they remain clinically unidentified, even though their presence is later manifested by the subsequent determination of antibodies in the blood, as shown by the results of wide investigations. Men and women are subject to the infection to the same degree, also children frequently become infected. The percentage of the immune group of the population in the endemic centers rises gradually to 70 - 80 for the age group 20 - 25, and then remains at this level.

RESTRICTED

in contrast to the classical yellow fever, transmittable by the *Aedes aegypti*, there occur in the tropical forests and adjacent areas analogous diseases, in the complete absence of the above carrier. These diseases are known under the name of yellow jungle fever. The yellow jungle fever, in its clinical and serological aspects, is no different from the classical yellow fever, transmittable by the *Aedes aegypti*, but its epidemiology is different. This epidemiologically peculiar type of yellow fever became known in 1924, when its outbreak in Brazil was investigated.

In contrast to yellow fever, transmittable by the *Aedes aegypti*, the principal sufferers from the yellow jungle fever are adults captured in the forests. Apparently, the natural reservoir of the virus are animals, since none of the five antibodies in their blood, and cases of precipitation of the virus from wild mammals, captured in the jungle in June and August, are on record. This was the first proof of the natural contamination of wild animals with the virus of yellow fever.

Immunity, prophylaxis, and cure. Upon recovery from even a mild attack of yellow fever, a lifelong immunity is acquired. Children, born in the epidemic zones, and adults, living for long periods in these zones, are usually immune to yellow fever, with the immunity acquired as a result of a clinically unnoticed case of the malady having been endured. Virus neutralizing antibodies are discovered in the blood of people even 30 - 40 years and more, after having endured the disease.

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Since the patient is the source of contamination, the natural prophylactic measure is the thorough isolation of the stricken people. In cities and rural areas, where yellow fever is linked with mosquitoes as vectors, anti-mosquito measures are effective. However, in the case of yellow jungle fever, anti-mosquito measures are not applicable [for reasons stated above], and, therefore, prophylactic vaccination is resorted to.

A live vaccine, prepared from a virus culture on growing chicken embryos is used. The virus when utilized for this purpose, after having adapted itself to the embryo, lost its pathogenic characteristics for embryos, but retained the ability to induce encephalitis in mice through intracerebral contamination. The vaccine is introduced through a one-time subcutaneous injection. Various therapeutic methods were tested, but without success. Specific serotherapy, in particular, turned out to be a failure.

Laboratory diagnosis. The laboratory diagnosis of yellow fever is based on the precipitation of the virus from the patient. For this purpose, during the first 3 days of the illness, 5-6 mice are inoculated intracerebrally with the blood of the patient, with subsequent passage of the virus and the histological examination of their internal organs, such as the liver, the spleen, and the kidneys. Serological examinations produce positive results, during the first days of convalescence, through the complement fixation tests. The virus neutralizing antibodies are discovered through neutralization tests on mice, beginning with the first weeks after the recovery of the patient, and during

RESTRICTED

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may have to follow, the latter test is the one that serves for the retrospective diagnosis of the disease.

The Virus of Dengue Fever.

General data. Dengue fever is an acute infectious disease occurring in tropical and subtropical areas. It is characterized by a sudden appearance of fever, headache, aching muscles, leucopenia, and a rapidly passing exanthematous rash. The disease usually ends in recovery by the 6 - 7th day.

Precipitation of the virus. It was established for the first time in 1907 that the inhibitor of dengue fever is a filovirulent virus transmitted by mosquitoes (Aedes aegypti).

Pathogenic characteristics. The majority of the laboratory and also tested turned out to be non-susceptible to this virus. In some cases, the virus was observed and isolated during 5 days after contamination. Certain species of primates are susceptible to the virus, and they become ill with mildly pronounced symptoms.

Experiments with exclusively virulent virus stems of Dengue fever, precipitated during the epidemic in Hawaii, were reported recently. Human serum, contaminated with this stem, contained 1,000,000 infectious doses per one cubic centimeter, and, by centrifuging, this concentration of the virus was increased tenfold. By the use of this concentrated material, 16 passages of the virus through intracerebral inoculation of 2 - 12 day-old white mice, were effected only 10 to 20 percent of the contaminated mice showed symptoms after 3 - 4 weeks of incubation, but, beginning with the sixth passage, the incubation period

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became lower, and the percentage of animals manifesting symptoms of the malady, became higher. With the 11th passage, 10 out of 20 three-day old mice, and only one out of 10 six-weeks old mice, were stricken out of 4 virus stems, precipitated in New Guinea, only one turned out to be pathogenic to mice. A virus, after having been passaged through mice, is known to have induced the Dengue fever in humans, but with further subsequent passages, it was losing its infectious characteristics.

Size, filterability, stability, cultivability. The virus passes through Berkefeld and Chamberland 17 and 113 filter candles. In the serum of patients, when preserved under various conditions at 15 - 18 degrees Centigrade, the virus remains viable for 50 days. In a desiccated state, it remains viable for 3 months and over. The Dengue fever virus can be cultivated on the chorio-allantois of a chicken embryo. There are no crossover reactions with the virus of yellow fever.

Epidemiology. The disease is known in countries and areas having a warm climate, it originates during the summer months, and is frequently manifested by epidemic outbreaks involving large groups of the population. The malady is non-contagious, and is transmitted by several species of mosquitoes. The principal carrier is *Aedes aegypti*. It was established that the patient can infect the mosquitoes even during the incubation period, and the blood of the patient is infectious during the . . . first 3 days of the morbid period. After filling up with such blood, the mosquitoes can transmit the infection through stinging after 3 - 11 days, and still remain infectious for the rest of their lives.

524
RESTRICTED

RESTRICTED

1. Dengue Fever, Epidemic. The epidemic form of the disease is, essentially, a result of the infection having been endemic in the past. The presence of specific antibodies and their part in the immunity are not yet firmly ascertained. There also are no effective vaccines, or other specific prophylactic or therapeutic means. A live vaccine, prepared from the virus modified by intracerebral passages on mice, induces a short-lived immunity, and has no practical significance. Prophylaxis in the dengue fever is based on anti-mosquito defense in general, and individual protection from mosquitoes by the use of netting and repellents.

The Virus of Paparangi Fever.

General Data. The Paparangi virus, similar to the dengue fever, is an acute infectious disease, induced by a filterable virus and transmissible by the biting of a blood-sucking carrier. The disease comes up suddenly, after 1 - 6 days of incubation, the feverish phase, accompanied now and then, general asthenia, headache, and muscle aches, lasting from 3 to 5 days. The typical indicator of the disease is hyperemia of the face, conjunctiva, and pharynx. The body temperature rises to 39 - 40 degrees Centigrade and, toward the 3rd day, descends lytically to normal, the pulse constantly lagging behind the temperature. The tongue is coated with a brownish film, feels dry, with the appearance of frequent cracks. The uvula becomes edematous, and at its base, at times, exanthema is observed. Frequently, the malady is accompanied by gastrointestinal disorders. Recovery usually follows the end of the feverish phase. However, a second

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and third method of fever may occur.

Reproduction of the virus. In 1941-42,

Lepp, et al., established the virological nature of the malarial fever and the fact of the infection being transmitted through the bite of the mosquito *Anopheles gambiae*. To date, no susceptible animal that could be infected experimentally with malarial fever has been found. Only monkeys (*Macaca mulatta*) are highly susceptible. Rhodesian and Natalian. In 1943, infected rabbits with the virus of malarial fever through a subcutaneous injection. The rabbits responded to the subcutaneous inoculation with the blood of malarial patients by developing the typical temperature reaction. The urine of these rabbits, but to death at the height of the fever phase, contained the virus capable of infecting humans.

Size, ultrastructure, and resistance of virus. There is no

generally accepted opinion as to the size of this virus. Some consider its size to be 160 millimicrons, others - 20 - 160 millimicrons. It passes through Berkefeld B, Chamberland L₃, L₅ to L₁₅ filter-coalesces. It becomes inactivated at a water bath temperature of 56 degrees Centigrade within 10 minutes. Oil kills the virus. Blood, containing the virus, remains infectious at room temperature for 3 - 7 days. With the addition of 50 percent glycerin, citrated blood retains its infectious characteristics up to 14 days. In a dehydrated state, the virus can be preserved for months.

Virus cultivation. The most successful method is the cultivation of the virus on the chorioallantoic membranes

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of chicken embryos, with intensive proliferative transmutations by feeding on the membrane. The cultivation of the virus by contaminating the growing chicken embryos into the yolk sac is also successful.

Epidemiology. Pappatasii fever, in addition to tropical and subtropical countries, is widespread along the entire Mediterranean coast. In the USSR, it occurs in the Crimea, in the Caucasus, and in Central Asia. Mosquitoes are the principal custodians of the virus in nature. The transovarian transmission of the virus in mosquitoes has been proved. Meshkovskiy and his collaborators established the fact that during inter-epidemic periods the virus is preserved in hibernating mosquito larva. During the epidemics, the basic source of infection to the human, who contaminates the mosquitoes. It is possible that, in some cases, humans are playing the part of virus carriers over prolonged periods, since recurrent attacks of the malady upon people, after they left the endemic centers, are on record. It is also possible that the theory natural endemicity (Pavlovskiy) of some transmissible infections is also applicable to Pappatasii fever, since cases of the disease occurring in humans, during their penetration into virgin areas, are on record (Litvinsky).

The summer seasonality of the malady and the epidemic peaks during the hot weather coincide with the accelerated propagation of the carriers during the same period.

Immunity, Prophylaxis, and cure. People, who have recovered from Pappatasii fever, acquire an immunity, the duration of which is not yet clearly established. It is most probable that the immunity lasts only several months. However, in some

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...and, antibodies were discovered in the sera of some people
 a year after they have entered the galaxy. With the aid of
 the Kanyan tests, the possibility of discovering the role
 of the antibodies was established. A prophylactic con-
 sideration of preventing specific diseases and measures
 for prophylactic intervention. Victims of prophylactic vaci-
 nations have not, as yet, acquired any practical significance.
 And since the alien people are the source of the infection,
 it becomes necessary, for prophylactic reasons, to isolate
 all patients from any possible contact with hostiles.

The Virus of Venereal Lympho-Granuloma.

Veneral Lympho-Granuloma is first de-
 scribed as an infectious venereal disease by Neisser and Carr,
 and is frequently called by the name of these scientists, on
 the fourth venereal disease. Morbidity is manifested at first
 by general symptoms: high temperature, rigor, vomiting, asthenia,
 aches in the joints, and, finally, the development of adenop-
 athy of the lymphatic glands, frequently with bilateral localiza-
 tion. The nodes of the greatly enlarged glands are frequently
 softened and the accumulated purulent matter precipitated through
 the forming fistula. The slow precipitation of the pus through
 each fistula is conducive to the protracted chronic course of
 the malady. Frequently there is a diffuse affection of the
 entire lymphatic system of the patient. Cases are on record,
 where various ~~organs and systems~~ and, at times, the entire central
 nervous system are drawn into the process of infection. There
 are cases of keratitises, induced by the virus of venereal

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in the granuloma. An inflammatory condition of the rectum occurs frequently.

The histological changes observed in the glands do not constitute an index, which is specific for this infection. The subacute granulomas being formed are frequently similar to the multifarious abscesses in tuberculosis or syphilis. In the early stages, pinhead-like epithelioid formations, disseminated along the gland, an abundance of mononuclear cells, at times, eosinophiles and neutrophils, may be observed. Later on, necroses, surrounded by epithelioid and multinuclear cells, appear. The monocyte-phagocytosis of pyknotic nucleolar material is typical. Subsequently, the histological picture becomes more typical, and in the late stages of the disease it becomes useful for diagnostic purposes. A clearly pronounced plasmacellular infiltration may be noticed during rectal changes in the tissues, while abscesses and granulomas are observed rarely.

During the early, acute phase of the disease, the blood shows slight leucocytosis with relative mononucleosis. The velocity of erythrocyte precipitation is increased.

Preparation of the virus. The virusological nature of the disease was finally established only after the investigations of Hallectren and Wassen. In 1930, they established the fact that the material, taken in an aseptic manner from the lymphogranulomatous nodes of a human, and inoculated into the brain of a monkey, induces in the latter a process of infection. A multitude of various virus stems, which were precipitated subsequently turned out to be similar to each other, and

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are maintained through intracerebral passages not only on monkeys, but also on white mice.

Pathogenic characteristics. The virus is highly pathogenic to anthropoid apes (chimpanzee), certain species of lower apes, dogs, cats, white mice, and mildly pathogenic to rabbits and pigs. The intracerebral inoculation of the infectious material, after 6 to 12 days of inactivation, induces in susceptible animals meningoencephalitis with sharp clinical manifestations, resulting in death. Clinical recovery is observed rarely, and even then the animal remains a carrier for a long time. Some animals enter the disease without showing any clinical symptoms, and only characteristic histological lesions in the central nervous system and the presence of small virus concentrations in the brain are proof of the infection. The clinical condition of symptoms of meningoencephalitis, which is similar to that in humans, can be induced by contaminating monkeys with the adapted virus stem via the endogenous ingredients of the sex organs. Cats can be contaminated intracerebrally, with the clinical aspects of the disease frequently similar to the same in monkeys. Mice, inoculated intracerebrally, are stricken on the 6 - 12th day with encephalitis and, frequently, conjunctivitis. They can also be regularly contaminated intranasally, with the resulting large accumulation of the virus in the lungs. The virus can be discovered in the blood and lymph of susceptible animals during the first few days of the infection, regardless of the contamination method.

Filterability, size, morphology. The virus of venereal

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Lymphorandio passes through L1, L2, and Chamberland L2, L3 filter-bundles. By differential ultracentrifugation, its size was established to range between 120 and 165 millimicrons, and by filtration - from 125 to 175 millimicrons. Thus, this virus belongs to the group of the largest viruses. This makes for a successful study of the morphological structure of the virus, appearing in the form of elementary corpuscles in heterogeneous infectious material. The lymphorandio atoms elementary corpuscles are discovered in or around formations in the large endothelial cells.

The morphological variability of the elementary corpuscles was described in detail by Chen (1936 - 1939). Chen points out that, parallel with the development of the infection and the reactive changes in the stricken organ, the corpuscles lose their clearly outlined roundness, are slightly elongated, begin to stain with less intensity of elective axes, and gradually disappear from the cells, in which they were discovered. By the empty vacuoli remaining in the cells, the disposition of the vanished accumulations of the lymphorandio atoms corpuscles can be determined. "In some cases, the agglutination of the corpuscles occurs, as a result of which intracellular compact formations of various sizes are created" (Chen, 1936-1939).

Recently, the existence of a toxicant, linked to the elementary corpuscles, was established. The toxin is precipitated by prolonged centrifuging at 15000 RPM, and, together with the corpuscles, is retained on the collodion filter-diaphragms. Mice inoculated with this toxin intravenously or

RESTRICTED

or intraperitoneally die within 4 - 20 hours. This toxin can be neutralized by a specific antitoxic rabbit serum.

Cultivation. The virus can be successfully cultivated only by contaminating a receptacle of agar or into the yolk sac, then succeeded in cultivating the virus in all plant inoculated tumors, and even successfully maintained the virus inside of normal passages in tumors.

Stability. The virus is preserved and maintained in the laboratory by passages on mice and monkeys. When vacuum-dried, the virus will survive for many months. In contrast to other viruses, the virus of lymphogranuloma at sea cannot be preserved in dry form. It will survive for 2 weeks at 4 degrees, and for over a month at 10 - 20 degrees centigrade. Higher temperatures cause the rapid inactivation of the virus: at 30 degrees, within 24 hours, at 36 degrees, within 20 minutes. Ultraviolet irradiation and the photodynamic effect of methylene blue inactivate the virus within 5 - 10 minutes.

Differentiation of the virus. By morphological and serological investigations, the similarity of the virus of venereal lymphogranuloma with the viruses of psittacosis, meningopneumonia in mice, trachoma, and infectious conjunctivitis was established. With the tissue albumin washed off, the corpuscles become agglutinated under the effect of specific serum antibodies. Shen points out that the histopathological changes in experimental lymphogranulomatous encephalitis allow, in well defined cases, the microscopic differentiation of this infection from other virus encephalitis in mice. These peculiar characteristics consist of a sharp meningeal infiltration, perivascular lesions, mani-

tested in the appearance of lymphoidal nodes resembling the proliferation centers of the lymphatic glands, in the selective lesions of the ventricle walls with excessive dilation of the ventricular cavities, and similar phenomena of hydrocephalia. In the spinal cord, in addition to inflammatory phenomena in the meninges, lesions in the nerve roots, with peri- and endoneuritis, are observed.

Epidemiology. The infection is known practically throughout the world, but is widespread in Sweden, Germany, Russia, Italy, France, the United States, Greece, Africa, Indo-China, Japan. In the Soviet Union, only individual cases of lymphogranuloma are described. The infection is transmitted by direct contact. The gateway of the infection are excoriations on the integuments of the sex organs, with contamination possible not only through contact with acute open adenitis, but also through contact with its chronic form and through clinically healthy virus carriers.

Immunity, prophylaxis, cure. After having endured venereal lymphogranuloma, a person rapidly develops an immunity, and in the serum from patients and convalescents are found complement fixing and virus neutralizing antibodies. A specific immunological, or allergy tests, suggested by Frey in 1925, makes the timely exposure of virus carriers and persons with dilated forms of the infection possible, preventing thereby the further dissemination of the disease. The Frey test runs essentially as follows: an antigen, containing the pus from a lymphogranulomatous bubo a 1:10 dilution, is heated at 60 degrees Centigrade for 2 hours the first day, and for one

hair the next day, and then, in a dose of one cubic centimeter, injected subcutaneously. The skin reaction, at the antigen injection spot, is in evidence after 4 days, and is considered positive, when manifested in the form of an erythematous zone .5 centimeters in diameter, sometimes with a necrotic nucleus in the center.

The obtaining of skin and infectious material from patients being ill, it was suggested that, for intracerebral testing antigens, prepared from the brain of contact-infected monkeys and mice, be used. The antigens, prepared from the lungs of sickened mice and, as recently introduced, from the virus culture in the yolk and of a chicken embryo, turned out to be particularly effective. The percentage of positive Frey reactions in repeated intracerebral tests, during the development stage of the lymphocytic choriomeningitis virus, frequently reached from 70 to 95. In the early stage of the disease, the reaction may be negative. It was noted that the pre-allergic period may be rather prolonged.

The clinically manifested, as well as the dilated forms of the disease, diagnosed with the aid of the Frey reaction, and also the exposed chronic virus carriers, can all be successfully cured by the use of sulfamide compounds. Beginning with 1938, the timely identification of the infection and the wide application of sulfamide compounds in the therapy of its various forms, have considerably reduced morbidity.

Laboratory diagnostics. The laboratory diagnosis is based on the Frey reaction and also on the intracerebral con-

infection of mice and monkeys. In addition, there is the search for elementary viruses under the microscope, the complement fixation test with sera from patients, virus neutralization test with sera from convalescents.

3. Infection of animals for the obtaining of diagnostic sera is important, since the immunity, formed in susceptible animals, is instantly and short lived.

Virus Diseases in Laboratory Animals.

Among conditions of virusological laboratory work, the existence of a series of infections, to which the participating animals are subject, must always be taken into account. It must be emphasized that these infections in laboratory animals were the cause of some failures and errors in the experimental study of the etiology of some virus diseases in humans.

Below, is a brief description of presently known viruses, detected during the latent phase of the disease.

The virus of encephalomyelitis in mice. In 1935, Taylor described a newly discovered virus disease of mice in America - spontaneous encephalomyelitis. Among seemingly healthy mice in the vivarium were found some with paralysis of the posterior extremities. A virus, pathogenic to mice only, was precipitated from the brain of these mice, and also from their intestines. In the USSR, the virus of spontaneous encephalitis in mice was precipitated by Solon'yev and Shadladze in 1940, and later on by Shifrin and Yablonskaya.

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White mice are highly susceptible to this virus through
intracerebral and intranasal contamination.

Clinically, the course of the disease in mice, infected
with the virus of encephalomyelitis, is similar to the disease,
induced in mice by the inoculation with the - Japanese or St.
Louis encephalitis. Following a 1 - 6 day incubation period,
there occur pareses and paralysis, most frequently of the poster-
ior, but sometimes also the anterior extremities, and after 1 -
3 days, the mice usually die. The morbid phenomena in mice,
whether stricken spontaneously, or contaminated experimentally,
are the same. In contaminations with the Taylor virus, the in-
cubation period may be drawn out to 30 days. In mice, which
have survived the infection, the virus may be preserved in the
brain up to 8 weeks.

The histopathological examination of the brain of
stricken animals disclosed meningoencephalomyelitis with
neuronophagia, which is particularly pronounced in the cells
of the anterior cornu of the spinal cord.

The virus passes freely through Berkefeld and Chamberland
candles and Zeitz filters. Its size, as established by filtra-
tion through gelatin-perforated diaphragms, is 13 - 19 millimi-
crons.

In 50 percent glycerin the virus can be preserved for 150 days
at 4 - 6 degrees Centigrade. Heating in a water bath at 60
degrees Centigrade kills it within 30 minutes.

The virus can be cultivated on the chorioallantois of

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a chicken embryo, and also by contamination of an 11 - 15 day old chicken embryo into the brain.

The neutralization and complement fixation tests are used in order to discover the specific antibodies.

The non-susceptibility of guinea pigs and white rats helps to differentiate this virus from the virus of lymphocytic choriomeningitis. The non-susceptibility of monkeys helps to differentiate this virus from the virus of human poliomyelitis. The viruses of tick - and Japanese encephalitis may show mild crossover reactions of neutralization with the virus of encephalomyelitis in mice, but the crossover-inhibitory test brings out the clear differences between the wild viruses.

Figure 52. [microphoto] Spontaneous encephalomyelitis in mice, induced by the Taylor virus stem. Lesions in the spinal cord. Medullary meningitis and myelitis. Stained as per Romanovsky - Giemsa (collection of the Institute of Virology).

Figure 53. [microphoto] Spontaneous encephalomyelitis in white mice, induced by the Taylor stem. Vascular lesions in the medulla oblongata. Stained according to the Romanovsky - Giemsa method (collection of the Institute of Virology).

~~The epidemiology of encephalomyelitis in mice~~ has, as yet, been studied very little. According to Taylor, cases of spontaneously stricken white mice occur only in a ratio of 1 : 2000. Paralysis are discovered most frequently in young mice, in the

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are 4-6 or 6-7 weeks. Contact contamination tests produced no positive results. A supposition was advanced about the possibility of the virus being transmitted to blood sucking insects, which are parasitical on mice. A considerable importance in the dissemination of the virus is the fact of its presence in the intestines of animals.

Stability to recurrent contamination is observed in mice recovered from the disease. Adult and old mice frequently show higher stability to contamination. Mice having endured the malady and having remained with residual paralysis, are also immune to recurrent intracerebral contamination. Immunity is accompanied by the accumulation of antibodies in the blood.

Virus of Encephalitis V in Mice.

The presence in monkeys of a neurotropic virus named virus V (Sabon and Wright, 1934) is known. This virus was precipitated after a lethal case of sickness of a human occurring as a result of having been bitten by a seemingly healthy Macacus rhesus. In addition to monkeys, rabbits and guinea pigs are susceptible to this virus. It seems that the contamination with virus V is widespread in monkeys, since, in the organisms of many of these animals, virus neutralizing antibodies are discovered. However, natural morbidity and lethality are rare.

The Virus III (Rivers) in Rabbits. There is a spontaneous disease of rabbits, called virus III (Rivers and Tillett, 1923). By intratesticular inoculation into rabbits, this virus induces fever, changes in the testicles and skin, with the formation of

RESTRICTED

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intracerebral injections in the epithelial and endothelial cells. These cytoplasmic inclusions are similar to typical inclusions. Inoculation intracerebrally into rabbits, the virus incubation period is 10-14 days. After the disease, rabbits acquire an immunity for periods of 6 months, and their serum contains virus neutralizing antibodies.

This virus has precipitated only intracerebral diseases of testicular tissue, and the symptoms for the spontaneous form still remain unknown.

The virus of poliomyelitis in guinea pigs.

Poliomyelitis, or the so-called infantile paralysis (Koch, 1921) can be transmitted to healthy animals through an intracerebral inoculation, or a lesion of spinal cord lesion. After 9 - 22 days of incubation, the guinea pigs become feverish and begin to lose weight, at the same time development of muscular atrophy and paralysis of the posterior extremities and, at times, of the bladder. The disease runs its course from 3 days to 3 weeks. The virus is found in the central nervous system, in the mesenteric lymphatic glands, and, sporadically, in the liver and spleen.

The virus in the salivary glands of guinea pigs. In 1926, Kolbe and Kuthner observed considerable edema in the epithelial cells of the salivary glands in adult guinea pigs. Intracerebral injections of a suspension of these stricken glands into healthy guinea pigs results in death on the 5 - 7th day, amid manifestations of diffuse subacute meningitis.

In cases of recovery, the serum from the animal shows an

RESTRICTED

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and relation of virus neutralizing antibodies.

Experimental infection of animals with virus.
It is necessary to note the existence of a disease in laboratory animals, which is caused not by a virus, but by a micro-organism homologous to the protozoan, and which under the name of amoebic borreliosis is well known in the brains of guinea pigs, mice and rats. There are usually no symptoms in spontaneous cases, but symptoms may appear, when this latent infection is provoked, for example, by intracerebral inoculation with any kind of infectious matter.

A histological examination of the brain of an animal shows solid inflammatory changes with marked consistency of a necrotic center, surrounded by epithelial and endothelial cells. In the centers of these nodules are oval cyst-like corpuscles containing minute spherical particles with circumferential granules.

Under natural conditions, amoebic borreliosis in animals is spread, as it seems, by direct contact.

The virus of infectious ectromelia in mice. This disease was first discovered by Marshall in 1930 among laboratory white mice. The stricken animals show edema of the paw, particularly from the underside, its surface becoming semispherical and slightly translucent. In the next stage there is a disruption of the skin integuments, the oozing of the serous liquid onto the surface, and the formation of incrustations. The stricken section of the paw is separated from the healthy part by a sharp

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For the preparation of histological slides from these sections, it is possible to use, in addition to the usual methods of fixation, a fixation reagent made up from 100 cubic centimeters of a saturated solution of mercuric chloride and 5 cubic centimeters of glacial acetic acid. The inclusions are readily stained by hematoxylin-eosin and other dyes.

...the virus is present in the skin of the stricken paw and in almost all internal organs, particularly in the liver. It is pathogenic to mice under various methods of inoculation. In

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experimental work, contamination into the skin of the under-side of the jaw, or into the abdominal cavity, is most frequently resorted to. Since the initial contamination material is full of interferons, it is necessary to keep it for 2 - 3 days in a 0.5 percent phenol solution, or to filter it through a large-pore filter, before using. The incubation period in mice, under conditions of experimental contamination, is 2 - 3 days.

The virus of ectromelia is one of the large viruses, its size having been determined as 135 millimicrons. The elementary corpuscles of the virus are easily exposed during the micro-examination of smears prepared from the skin of the jaws and from the liver, stained in accordance with the Papanicolaou method. They are minute round corpuscles, lying either within, or outside of the cells, and frequently forming large accumulations.

The virus can be cultivated in vitro in a medium containing pieces of embryonic tissue of mice, or on the chorio-allantois of a chicken embryo. After the virus was put through several passages on chicken embryos, it becomes pathogenic to rabbits and guinea pigs.

The virus is well preservable at low temperatures and is unstable to heating. At 55 degrees Centigrade it is inactivated in 30 minutes. It has stability to phenol, to the extent that in 0.5 percent phenol it remains active for 50 days, and in one percent phenol - for 20 days. A 0.01 percent formalin solution fully inactivates the virus within 48 hours.

The mink is very infectious for mice. If one stricken mouse finds its way into the vivarium, all healthy mice become contaminated. In experimental work with a diversity of material, such mice must never be used, since they are bound to perish of ectromelia, thereby confusing the results of the experiments. The virus of ectromelia may also be adapted to cerebra tissue, and, frequently, during the attempts at precipitation of a neurotropic virus from some material, the virus of ectromelia may turn up.

Figure 54. [Photo] Various stages in the lesion of the paw of a mouse stricken with ectromelia: (1) normal paw; (2) intensive edema; (3) the beginning of necrosis.

Figure 55. [Microphoto] Smear from the paw of a mouse stricken with ectromelia. Crushed inclusions with abundance of elementary corpuscles. Silver-stained by the Florey method. Magnified 1200 times (micropreparation by Turevich).

Figure 56. [Microphoto] Protoplasmatic inclusions in the skin on the paw of a mouse stricken with ectromelia.

To avoid the occurrence of epizootics of ectromelia, it is necessary to subject all mice arriving at the laboratory to a thorough examination. If, within a given shipment of mice, ~~no mice are~~ ~~swollen snouts~~, swollen snouts, or gangrenous tails are discovered, the entire shipment of mice is to be rejected. Animals suspected of coming down with any sort of

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malady, even though sympto-less for the time being, may be accepted for experimental work only after undergoing a two-week quarantine period, and this only on top of a thorough-going elimination of ectromelia by laboratory tests. The laboratory diagnosis of ectro-elementary corpuscles and the reproduction of the infection by intraplantar or intraabdominal inoculation of young mice.

In the serum of surviving mice occurs the accumulation of antibodies, detectable by the neutralization test. The inactivated virus does not create immunity and does not cause the accumulation of antibodies.

The elementary corpuscles, after the tissue has been removed by differential centrifuging, can be agglutinated by an immune serum. Solov'yev obtained a clear agglutination of the elementary corpuscles of the virus of ectromelia with a serum from immunized chickens. The reaction may be observed macroscopically.

The virus of ectromelia agglutinates the erythrocytes of chicken, the addition of immune serum inhibits hemagglutination.

In precipitating any new virus in the laboratory, ectromelia must first be eliminated. To accomplish this, several passages of the liver of externally healthy mice are to be effected on mice via intraabdominal and intradermal contamination.

Pneumotropic viruses in animals. There are known viruses, which cause pneumonia in cats, skunks, mice. We will dwell here only on such spontaneous maladies of white mice, since they have

-344-

RESTRICTED

RESTRICTED

been studied in more detail, and since they have a significant bearing upon the day-to-day laboratory procedures, where these animals are concerned. Seven viruses, precipitated from seemingly healthy mice as a result of serial passages of their lung tissue, are on record. These viruses fall into two groups. In the first group are viruses, which are morphologically similar to the virus of psittacosis and venereal lymphogranuloma, and are represented by elementary corpuscles of the same form and size. These viruses are not only similar to the above externally, but also have common serological links with them, on which basis they are considered to be members of a homogeneous group. All of them are, to a greater or lesser degree, subject to the effect of sulfonamide compounds.

The next studied representative of the second group of viruses of pneumonias in mice, is the virus precipitated by Andrews in 1945. This virus is pathogenic to mice, and only via intranasal inoculation, it induces in their lungs the formation of peculiar consolidation nuclei of a gray-red coloration and a sharp edge of the lung tissue. It is called the virus of gray pneumonia. It can survive, in a latent state, in the lungs of mice for $7\frac{1}{2}$ months after artificial contamination, and then effectively induce the disease in healthy mice, when they are inoculated with the above described lung tissue. A peculiar characteristic of this virus is that, in animals vaccinated with it, it does not induce the formation of antibodies and immunity. In addition to mice, only cottonfield rats are susceptible to this virus. In contaminating guinea pigs and rabbits, the virus is preserved in the lungs for a period

-345-

RESTRICTED

RESTRICTED

ing, for 13 to 37 days. Among mice, the infection is transmittable, as it seems, by direct contact only, with the degree of contagiousness of the infected mice, however, being rather low.

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-346-

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